Giving blood: Donor stress and hemostasis

Don't let your blood run cold

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Does a donation induce stress in blood donors, and does this affect the donor’s hemostasis? Indeed, the results presented in this thesis show the presence of a donation-induced psychological, hormonal and physiological stress response. Thereby, psychological and physiological stress responses indicate a decrease in stress from pre- to post-donation, while peaking during needle insertion and uncoupling. The donation-induced stress responses are associated with small but clear pro-hemostatic effects. In conclusion, a whole-blood donation induces stress in blood donors, thereby affecting the donor’s hemostasis.
GIVING BLOOD: DONOR STRESS AND HEMOSTASIS

Don’t let your blood run cold

Maurits D. Hoogerwerf
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DON’T LET YOUR BLOOD RUN COLD

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aan de Universiteit van Amsterdam
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Part I
Introduction
CHAPTER 1

General introduction

Ms. R., a healthy young woman, is not an experienced blood donor and, until now, has made only one donation. During that donation, however, she fainted. When receiving the second invitation card to donate blood, Ms. R. feels her heart beating. Again?

After a few days of thinking about it, she decides to go. Together with a close friend she plans a visit, even though they both have a lot of work. Unfortunately, it’s rather busy at the collection center, so that the hostess has little time for her. On the donor health questionnaire, Ms. R. indicated that she had fainted during the previous donation. Despite her nervousness, during the health screening she has a good talk about this with a pleasant donor physician.

Whilst waiting a few minutes in the donation area until a bed is available, she is anxious and looks curiously at the other donations being performed. A friendly nurse then performs a routine venipuncture, and remarks on the excellent accessibility of her veins.

After what feels like an eternity - but is in fact only a few minutes - needle removal takes place. On the previous occasion, this was the moment when she fainted. Again, she feels her heart beating and tension in her legs and shoulders. However, this time nothing happens and, after a successful donation, she is directed to the donor canteen. She grabs a pink biscuit and a cup of tea, and with a sigh of relief, sits down at a table.

Every day, people donate blood at blood collection sites around the world. At Sanquin (the Netherlands) each year about 343,000 blood donors voluntarily provide this blood bank with approximately 435,000 units of whole-blood and 285,000 units of plasma or platelets [1]. Blood units and their derived products are essential for modern healthcare as they are widely used in hospitals (e.g. during operations) and for the processing of drugs (e.g. against hemolytic disorders) [2, 3].
Chapter 1. General introduction

In recent decades there has been a slight increase in donor-related studies that have investigated, for example, the incidence and management of negative donation experiences, the recruitment and retention of donors, or the effect of negative experiences on donor return rates [4–7]. However, very few studies have explored donation-induced stress responses [8, 9]. Until now, the effects of these donation-induced stress responses on the donor’s hemostasis, as assessed in general stress literature [10], have largely been overlooked.

An introduction to stress

Before introducing stress in a donation-related setting, a brief overview of stress is presented and stress as a concept is defined. During the past century, researchers have developed different conceptual models to describe and understand the human stress response and its consequences [11–13]. The acute stress response was originally proposed as being a response to a stressor, such as confrontation with a dangerous entity, such as a large snake.

When exposed to such a (potential) stressor, an accumulation of different responses takes place, interacting with each other and, overall, increasing the total level of arousal or awareness. This cascade can be divided into three pathways: psychological stress, hormonal stress, and physiological stress. Psychological stress responses include increased levels of anxiety, fear or tension [14–16]. Hormonal stress responses include increased adrenaline and cortisol excretion [15, 17]. Physiological stress responses include increased heart rate, increases in systolic/diastolic blood pressure [18–20], and differential changes in heart rate variability parameters [20, 21]. In addition, changes in hemostasis can take place, leading to increased coagulation [22, 23].

These common stress responses are affected by several factors. One important factor known to add to a stress response is unfamiliarity with a situation [16]. Other factors influencing psychological and hormonal stress reactions are gender and levels of non-acute stress. However, depending on the type of challenge and stress reaction assessed, inconsistent and conflicting effects are reported for gender [24–26]. Non-acute stress (i.e. the wide range of daily hassles or minor daily pressures) is reported to enhance acute stress levels [27].

The cognitive activation theory of stress

The cognitive activation theory of stress (CATS) was proposed by Ursin and Eriksen in 2004, and their concepts are used in this thesis [15]. Briefly, their theory states that a stress response occurs when there is a discrepancy between what is expected or regarded as being the ‘normal’ situation, and what is happening in reality or is expected to happen. It is important to realize that, in contrast to previous stress
Theories, in the CATS a stress response may be triggered by both negative and positive situations, and is regarded as a general alarm, producing general and unspecific neurophysiological activation to face and deal with the new situation. The stress response implies non-specific changes as part of a general preparation to face any form of challenge or danger, by changing the behavior. Subsequently, the stress responses are reduced or eliminated when positive results of the actions are expected. When these expectancies are positive there is no health risk in a healthy organism. However, negative effects may arise when there is a lack of ability to deal with the situation. This has been defined as 'helplessness', i.e. the expectancy that there is no relation between response and reinforcement, and 'hopelessness', i.e. the expectancy that most or all responses lead to a negative result. When the behavior remains unchanged, both helplessness and hopelessness may eventually lead to disease.

**Stress and hemostasis**

As part of the stress cascade, physical activity and acute psychological stress are associated with a number of immediate effects in hemostatic parameters [10, 22, 23]. Studies on stress and hemostasis have described stress-induced increases in a number of clotting factors, e.g. factor VII, factor VIII and fibrinogen, as well as increases in von Willebrand Factor (vWF).

![Schematic overview of the coagulation cascade.](image-url)
Chapter 1. General introduction

Although often used interchangeably, hemostasis is defined as the process which causes bleeding to stop, and involves coagulation (or the clotting of blood) as the process by which blood changes from a liquid to a blood clot to end blood loss from a damaged vessel [28, 29]. This response might be due to a trauma damaging the vessel wall (e.g. an injury); however, as this process is part of the stress response, also physical activity or acute psychological stress can initiate onset of the cascade [10], starting within minutes after the onset of stress [22]. Although a detailed description of the entire mechanism is beyond the scope of this thesis, exploring some of the main processes seems relevant. In primary hemostasis, after injury of the vessel wall, circulating platelets in the blood adhere to the vessel wall with the help of vWF, and become activated so that they can aggregate and form an initial thrombus. Simultaneously, secondary hemostasis starts by activation of the coagulation cascade. The coagulation cascade comprises multiple components and a number of subsequent steps, amplifying the response. A schematic overview of the coagulation cascade is provided in Figure 1. As indicated by the dotted grey lines, the two pathways are separated, eventually joining together in a common pathway. The solid grey squares indicate the onset of the different pathways, whereas the solid black squares indicate the various factors involved. The black arrows indicate the activation routes of the pathways. In the extrinsic pathway, the exposure of sub-endothelial tissue factor initiates the cascade. Via a number of steps, this leads to the formation of factor Xa. In the intrinsic pathway, contact with negatively charged surfaces leads to activation of XII, which initiates a number of reactions eventually resulting in the formation of factor Xa. At this point, the intrinsic pathway joins the extrinsic pathway, leading to the formation of thrombin (factor IIa). Importantly, it is also thrombin that initially amplifies the whole cascade by activation of (among others) factor VIII. This causes amplifying feedback loops necessary to allow effective clotting. After its formation, thrombin initiates the formation of fibrin out of fibrinogen, which finally leads to the formation of a blood clot, e.g. on the site of injury.

Stress in blood donation

Since evidence suggests that acute stress might affect hemostasis, the potential of a donation-induced stress response is an interesting phenomenon. This stress response is influenced by the type of stressor, the specific situation, and the subject under stress. When transposed to a blood donation setting, the donation itself, or each step in a donation procedure, might elicit a stress response; however, the magnitude of this response is likely to be donor dependent. Thus, it is important to establish what is in fact known about this donation-induced stress response and its effects on blood products.
Donating blood

The first donor, Ms. R., has already been introduced. Now donor Mr. B. is presented, who serves to illustrate a different response pattern to a routine donation procedure.

Mr. B. is a healthy, middle-aged man, having made over 25 donations. Due to travelling, however, his last donation was some time ago.

After receiving an invitation card for a donation, Mr. B. decides to go immediately to the donation center, as, fortunately, he has that day off. After entering the center he is warmly welcomed by the hostess. There are no surprises on the donor health questionnaire, and also none during the health screening.

Because it’s not busy, during the preparation of the phlebotomy he has a pleasant conversation with the donor nurse. Insertion of the needle, withdrawal of the blood, and removal of the needle all go according to his previous experience. However, when he stands up he feels as though he is slightly faint. Luckily, this quickly passes, and he walks to the donor canteen to enjoy a cup of coffee and a waffle, and read a magazine.

In the Netherlands, a routine donation comprises a fixed order of steps: invitation letter, registration, health questionnaire, health and eligibility screening, phlebotomy, and refreshment area [30]. The stories of the two donors illustrate the variety in responses that might be shown to essentially the same procedure. Familiarity with the situation, indicated by donation experience (i.e. the number of previous donations) and previous experiences (e.g. adverse events), might influence the perception of experiencing the donation as being a stressful event or not, thereby shaping the subsequent stress response. Although a few studies have examined anxiety during a blood donation [8, 31], a systematic overview is still lacking, i.e. assessing the factors (e.g. donation experience) and associating them with psychological, hormonal and physiological stress reactions in blood donors in a blood donation setting.

Anticipatory stress response

Apart from a few studies reporting positive donation effects, such as a feeling of satisfaction after having performed this voluntarily activity [8], most of the literature in the field of blood donation describes negative donation experiences. These studies include the occurrence of a vasovagal reaction or bruise, the effects of negative donation experiences on donor return rates, and the interaction between the two [4–7]. For
instance, the presence of an adverse event has been shown to reduce return rates [4]. This effect differs between donors as, for example, men report less negative donation events than women, but are more likely to refrain from donating after experiencing one [5]. Because anticipatory stress is also known to cause (physiological) arousal [32, 33], donors who had a negative experience might show an increased stress response at the subsequent visit. As illustrated by Ms. R., feeling her heart beating, this increased anticipatory stress might be indicated by an increased pre-donation blood pressure. However, no studies have assessed this anticipatory stress response following a negative experience.

In addition, after having had a negative experience, the donor’s general anxiety and attitude to a future donation might influence the anticipatory stress response. Attitude is a measure of how the donor rates giving blood in terms of importance and pleasantness, and predicts the intention to donate in the future [34]. The influence of general, non-acute donation anxiety (i.e. how the donor rates the donation in advance in terms of fear) on donation intention is still unclear: some studies show no difference in intention [35] whereas others present evidence for a negative influence [36]. Although the effects of both factors on the intention to donate are under investigation, no studies have evaluated the effects of general anxiety and attitude to donating blood on the anticipatory stress response following a negative experience.

### Immediate donation-induced stress

The donation procedure itself might also be perceived as a potential stressor, and donation-induced stress responses have been described in a limited number of studies. Although overall results indicate an increased pre-donation psychological stress response, mostly only pre- and/or post-donation anxiety levels were assessed [8, 9]. In addition, potential differences in the donation-induced stress response throughout the donation procedure have not been considered. Studies evaluating hormonal and physiological stress responses are scarce and, thus far, provide inconclusive evidence [37]. Differences in donation-induced stress responses between gender and donation experience, or between levels of non-acute stress, have not yet been systematically investigated, whereas such factors can potentially influence the general stress response [16, 24–27]. Thus, in summary, there is a need for more detailed knowledge on donation-induced stress responses, comprising multiple stress measures at several key moments during a donation, and discriminating between the various groups of donors.

### Donation-induced effects in hemostasis

The main purpose of a blood donation is the provision of blood related products for clinical patients in need [2, 3]. This means that the quality of the derived blood products is of utmost importance and must meet quality criteria to assure patient
Giving blood: donor stress and hemostasis

As described above, acute mental stress is capable of inducing changes in hemostasis. Therefore, the question arises whether blood donation-induced stress responses are capable of inducing changes in hemostatic parameters, i.e. influencing the coagulation cascade, causing a higher clotting potential and possibly leading to undesired changes in the blood product. Although such an effect should be of interest in a blood donation setting, no research to date has investigated the potential effect of this donation-induced stress response on hemostatic parameters.

Donation-induced stress responses and their effects

To illustrate graphically the topic of thesis, a model was constructed encompassing the various elements related to blood donation-induced stress responses investigated in this thesis. This model is presented in Figure 2. As indicated by the thick arrows, central in this model is the concept that a blood donation procedure induces a stress response, which then has a number of potential effects. The stress response comprises psychological, hormonal and/or physiological stress reactions, which are thought to be influenced by a blood donation. Subsequently, these donation-induced stress responses might affect the immediate changes in hemostatic parameters. Donor characteristics play an important role, as stress responses might be influenced by characteristics such as gender, donation experience, levels of non-acute stress and the donor’s general anxiety and attitude to donating blood (thin arrow).

Figure 2 Conceptual model of the effects of donation-induced stress responses.
Chapter 1. General introduction

Aim, objectives and research questions

A routine whole-blood donation might be seen as a potential stressor and, thus, capable of inducing psychological, hormonal or physiological stress responses. The overall aim of this thesis is to provide detailed insight into the course of donation-induced stress responses and their effects on the donor’s hemostasis. This leads to the following objectives:

I Examination of the donation-induced psychological, hormonal and physiological stress response patterns during a blood donation procedure;

II Investigation of the effects of the donation-induced stress response on immediate changes in hemostatic parameters.

These objectives lead to the following research questions:

i What factors are associated with stress reactions in blood donors in a blood donation setting?

ii Do donors with and without a negative experience show different blood pressure levels at the pre-donation screening of the subsequent visit, and is this association influenced by the donor’s general anxiety and attitude related to donating blood?

iii Does a blood donation induce psychological, hormonal and physiological stress in whole-blood donors, and are there differences between men and women, first-time and experienced donors, and donors high or low on non-acute stress?

iv Does donation-induced stress have an immediate effect on hemostatic parameters in whole-blood donors?

Outline of the thesis

The remainder of Part I of this thesis is dedicated to the first objective, i.e. the evaluation of donation-induced stress responses. Chapter 2 presents a systematic literature review performed to explore research question i, to identify which factors (e.g. donation experience) are associated with psychological, hormonal and physiological stress reactions in blood donors in a blood donation setting.

In Part II, research question ii is investigated by examining whether donors with and without a negative experience show anticipatory stress at the subsequent visit (Chapter 3). This effect is evaluated by blood pressure levels at the pre-donation screening of a visit following a negative experience. In Chapter 4, this effect is further considered by assessing the role of general anxiety and attitude to donating blood among new donors.
In Part III, blood donation-induced psychological, hormonal stress, and physiological stress response patterns in whole-blood donors are explored in Chapters 5 and 6, to investigate research question iii. Differences between men and women, first-time and experienced donors, and donors with high or low non-acute stress are investigated and evaluated.

The second objective is studied in Part IV of this thesis. In Chapter 7, the effect of a donation-induced stress response on hemostatic parameters is assessed to answer research question iv.

To conclude the thesis, Part V provides a general discussion, in which the main findings are summarized and interpreted (Chapter 8). Various methodological and theoretical considerations are discussed, and implications and recommendations for future research and blood bank practices are presented.
Factors associated with psychological and physiological stress reactions to blood donation: a systematic review of the literature

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Blood Transfusion, 2015; 13 (3): 354-362
Abstract

Background: Maintaining a constant supply of high-quality donor blood is essential for society. However, adverse reactions are shown in 10 to 20% of the donors. Stress reactions elicited, might prevent donors from donating. Therefore, insight is needed in the factors associated with psychological and physiological stress reactions in blood donors around the blood donation procedure.

Study design and methods: A systematic review was performed by applying a sensitive search to the electronic databases PubMed, EMBASE and PsycINFO. Key words were terms related to blood donation, terms associated with psychological and physiological stress reactions, and both specific and non-specific terms used to relate possible contextual factors to stress reactions.

Results: The search resulted in 2,913 articles. After a screening procedure, ten studies were included. Psychological stress reactions included increased levels of pre-donation anxiety, fear and arousal. Physiological stress reactions included higher pre-donation heart rate, blood pressure and cortisol levels. Increased stress reactions were found to be associated with fewer prior donations, and both psychological and physiological stressors. Psychological stressors included high-distraction television, habitual anxiety and negative expectations. Also, coping strategies are thought to influence stress reactions. Physiological stressors comprised donor related symptoms, e.g. bruises and pain during the venipuncture.

Conclusion: Nine factors were found to influence the psychological and physiological stress reactions exhibited around a blood donation. Anxiety as stress reaction was assessed as a stress reaction in nine studies. A higher stress reaction was associated with fewer prior donations (seven studies) and physical symptoms (two studies).
Introduction

Blood donation is generally seen as a safe, voluntary and socially useful activity, based on the altruism of donors [40]. A number of positive effects of blood donation are described in scientific literature, such as feelings of satisfaction, feelings of being more alert and feeling better in general [8]. However, the majority of the literature concerning blood donation describes adverse events, such as fatigue, vasovagal symptoms, fainting and bruises [8, 41–43]. In addition to the discomfort experienced by the donor, these might also impede blood donation in the future. Studies report that up to 10 to 20% of donors experience such adverse events [5, 44]. For a blood supply organisation, it is crucial to maintain the donor pool in order to be able to deliver an optimal supply of blood, maintaining the donor pool is crucial. This can be achieved by recruiting new donors and retaining existing ones. Either way, negative donor experiences should be kept to a minimum.

In blood donor research, several studies have investigated the occurrence of adverse events, retention, or the interaction between adverse events and retention. For instance, Bravo et al. investigated factors associated with fainting and donating blood and showed that vasovagal reactions are more common during or after phlebotomy than during registration [45]. Newman et al. showed that donors who experienced an adverse event were less likely to return to the blood centre [4]. In line with this, Veldhuizen et al. showed a strong association between reporting a vasovagal reaction and stopping donating, especially among men [5]. Adverse events may upset the donors, causing increased anxiety. However, despite some indications that anxiety is increased before and during donating blood [9], it remains largely unclear what factors are associated with such a stress reaction.

Stress reactions are quite common phenomena [15, 46]. Various factors, known as stressors or stress stimuli, can induce a stress reaction. Although any situation and any object may elicit a stress reaction, the resulting stress experience may differ between individuals and circumstances [15]. After being confronted with a stressor, a psychological stress reaction occurs, which can consist of higher levels of anxiety, irritation, fear, worry, tension or anger [14]. At the same time, physiological stress reactions take place, such as increases in the levels of cortisol and (nor-)adrenaline [47], a decrease in heart rate variability [48], and negative changes in blood coagulation parameters [10]. As mentioned earlier, blood donation does not always elicit positive feelings, but can be accompanied by anxiety-eliciting factors as well [45, 49]. These factors (stressors) may induce a stress reaction.

The relation between blood donation on the one hand, and anxiety and stress reactions on the other remains largely unclear. Although stress reactions (e.g. anxiety) do seem to be present, the factors capable of inducing or enhancing these reactions (i.e. stressors) are largely unidentified. The aim of this study was, therefore, to perform a systematic review of the literature, in order to evaluate existing knowledge on the following research question: what factors are associated with psychological and
Chapter 2. Factors for stress reactions in blood donors

physiological stress reactions in blood donors in a blood donation setting? We hypothesised that both physiological (e.g. increased levels of adrenaline or cortisol) and psychological (e.g. increased levels of anxiety or fear) stress reactions take place in a blood donation setting. We also hypothesised that a greater stress reaction would be associated with factors such as donation-related adverse events, such as bruises or fainting, as opposed to not experiencing such an event. Knowing and identifying potential stressors could help optimise the donation experience for the donor.

Materials and methods

Information sources and search

One author (MH) prepared the search strategy in collaboration with two librarians. The strategy was discussed and adjustments were made resulting in consensus among all three individuals. The search strategy was then applied to the electronic databases PubMed (1948- June 26th, 2013), EMBASE (1980-June 26th, 2013) and PsycINFO (1806- June 26, 2013). Keywords differed per database, but generally included:

- terms related to blood donor (e.g. blood dono*) and blood donation (e.g. blood dona*).
- terms associated with psychological and physiological stress reactions, e.g. stress, distress, anguish, anxious, coping, avoidance, attention, arousal, emotion, fear, anxiety, nervous, worry, tension, irritation, cortisol, (nor-)adrenaline, heart rate (variability), respiration rate, blood pressure, pulse pressure, galvanic skin response, electrodermal activity, catecholamine, glucocorticoids, autonomic nervous system, orthosympathetic, (para-)sympathetic, HPA axis, thrombocyte, blood platelets, blood coagulation, prothrombin, platelet aggregation, platelet activation.
- both specific and non-specific terms used to relate possible contextual factors (stressors) to stress reactions, e.g. faint(-ing/-ness), dizziness, orthostasis, lightheaded(-ness), fatigue, adverse event, (pre-) syncope, vasovagal, haemorrhage, haematoma, contusion, bruise, nausea, sickness, deferral, phlebotomy, venipuncture, (risk) factor, determinant, marker, symptom, sign, precursor, effect and relation.

Both free text words (limited by title and abstract) and index terms were used to capture the topic of interest. The reference lists of the identified articles were checked for additional articles. Full details of the search strategies are available from the authors.

Study selection

Two inclusion statements were formulated and applied to title, abstract and whole text: (1) the article contained primary research outcomes in healthy blood donors
and (2) the study examined the effect of a potential factor on a stress reaction. If an article did not fulfil both statements, it was excluded. The retrieved references were selected by title and abstract by one reviewer (MH) based on the inclusion criteria. To check reproducibility, a random selection of the retrieved records was taken and screened by a second reviewer (IV). The results of this selection, including cases of doubt, were discussed (by MH and IV) until consensus was reached. After data extraction, inclusion of the remaining articles, based on the inclusion criteria, was discussed by all Authors.

Data extraction

The following data were extracted from the articles included: author, year of publication, country, description of the study population (sample size, age, gender, number of prior donations), donation type (whole blood, plasma), timing of the measurement of the stress reaction (before/during/after donation), stress reactions, stressors and main results.

Quality description

A quality description of the studies was performed by using items from guidelines on reporting and evaluating studies (i.e. the STROBE Statement [50], the Cochrane criteria [51] and guidelines for reviewing aetologic research [52]), and relevant items as reported in earlier review studies from our department, investigating stress in humans [53, 54]. For each study, two authors (JS and MH) independently judged whether or not the study described details about its participants, the methods and the results. The details required concerning the participants were the population from which the participants were drawn and the main characteristics of the study population (age, gender, number of donations), if applicable per study group. For the methods, details were required of a seemingly valid assessment method (e.g. timing of the measurements, name and reference of assessment instrument used) for both factors and stress reactions and a description of the appropriate statistics was necessary. The key results had to be summarised with reference to the study objectives. Disagreement between the reviewers was discussed until consensus was reached. The main objective of the quality description was to assess whether all items considered relevant to our review were measured, analysed and described.
Chapter 2. Factors for stress reactions in blood donors

Figure 1 Flow chart of studies in- and excluded in the different stages of the review process.

Results

Number of hits

After combining the identified articles from the different sources and excluding duplicates, the search strategy provided a total of 2,913 publications. The majority of the citations were excluded on the basis of title or abstract by the first author (MH). This step was replicated in a random sample (30%) by a second reviewer (IV) to check reproducibility. Differences and cases of doubt (<3%) were discussed until consensus was reached. The remaining articles (n=22) were fully extracted. Finally, ten studies were included in this review, based on the inclusion criteria applied on the full text and discussed by all authors. A flow diagram of the inclusion strategy is presented in Figure 1.
Quality description

Details from the quality assessment of the ten studies included are shown in Table I. All studies described the population from which the participants were taken. However, two studies did not present sufficient information about the donors’ characteristics. In four studies, insufficient details were available on the measurement of the stress reaction or the stressor. In these studies, although the stressor and reaction were measured, the timing of the measurements and/or the name and/or details of the assessment instrument were not presented.

Study descriptives

Detailed descriptives of the ten studies included can be found in Table II. The ten studies were published between 1984 and 2009. Seven studies were observational, one study described itself as being quasi-experimental [55], one as being a field experiment [56], and one study was originally set up as a randomised clinical trial [57]. Because the control group of this last-mentioned study was well described, this study was included and the results presented are those of the control group. Only an early study by Basler et al. used participants undergoing a cell-separation as a subgroup, the rest of the studies were based on (whole) blood donation [58].

Both physiological and psychological stress reactions were assessed in a blood donation setting. Psychological reactions were assessed in nine out of the ten articles [9, 55–62]. Three of these studies combined physiological measurements with psychological parameters [56, 59, 60]. One study focused only on the physiological stress reaction [37]. The results are shown in Table III.

Stress reactions

Psychological reactions that were assessed included levels of anxiety before, and/or during and/or after donation [9, 55, 57–62], stress [58], fear [56] and arousal [55]. Pre-donation stress levels of anxiety or fear were found to be higher than post-donation levels in all studies [55–58, 61, 62]. In addition, one study found higher anxiety levels during the actual donation compared to before and after donation [57], and one study showed that stress levels immediately before venipuncture were higher [58]. Two studies found a non-significant decrease in anxiety levels from before donation to during donation, whilst post-donation anxiety levels were not assessed [59, 60].

Physiological stress reactions were measured in four studies, which showed heart rate was higher before donation than after donation [37, 56], heart rate was higher before donation than during donation [59], higher blood pressure before donation than after donation [37, 56, 61] and/or higher pre-donation cortisol levels than post-donation ones, with a lower pre-donation cortisol in the fourth donation compared to the first
## Table 1  Quality Description.

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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ulrich, 2003 [56]</td>
<td>+</td>
<td>+/-</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Byrne, 2005 [61]</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Ditto, 2006 [9]</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hanson, 2009 [57]</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = described in the article  
+/- described adequately, although not in full detail  
- = not described in the article
Giving blood: donor stress and hemostasis

donation [37]. In these cases, blood samples for cortisol assay were collected 15 minutes before and 30 minutes after initiation of the phlebotomy.

In one study anxiety ratings were associated with the likelihood that a donor would return [9]. In women only, a negative association between these two was shown, in which female donors who exhibited higher levels of anxiety were less likely to return.

Stressors

A number of different factors were associated with stress reactions in a blood donation setting. Except for the number of prior donations - which was found to be relevant in seven out of ten articles [37, 55, 59–62] - most factors were found in two studies at most.

Prior donations

A factor associated with stress reactions during blood donation was the number of prior donations. In seven studies, inexperienced donors showed significantly higher levels of stress-related reactions than more experienced donors [37, 55, 58–62]. In the study by Basler et al., designed to assess differences in the donor strain between thrombocytapheresis and whole-blood donation, the authors found a significant negative association between stress and the number of prior donations for thrombocytapheresis donors only [58].

Psychological stressors

In total, five different psychological stressors were associated with stress reactions in a blood donation setting, and included character traits, television, music, alexithymia and coping method [55, 56, 58–61]. Coping methods, as studied by Kaloupek et al., were considered as a trait that influences the stress reaction exhibited [59, 60]. The authors showed that subjects making use of an avoidant or a problem-focused coping method exhibited lower stress levels both before and during donation. In contrast, a behavioural or emotion-focused coping style was associated with increased anticipatory stress reactions. Furthermore, donors who had given more prior donations were shown to make more use of an avoidant or problem-focused coping method, which resulted in lower stress reactions. In inexperienced donors a strong association was found between emotion-focused coping and anticipatory stress reactions.

Basler et al. studied several character traits and found that increased levels of habitual anxiety, more negative expectations and more extraversion were associated with significantly higher levels of stress around donation [58]. In a more recent study by Byrne et al., higher levels of alexithymia - a person’s inability to experience, express, or describe emotions - were significantly associated with higher pre-donation and
Table 2  Study descriptives.

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Country</th>
<th>Study type (design)</th>
<th>Sample size (gender a)</th>
<th>Age (year, mean±sd b)</th>
<th>Donation type</th>
<th>Prior donations (mean±sd b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basler, 1987 [58]</td>
<td>Germany</td>
<td>Observational</td>
<td>Test group 76, Control group 45</td>
<td>23.4±5.3</td>
<td>Blood</td>
<td>20 FTD d, 53 multi time</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Test group: 76 used cell-separator of which 33 were re-examined at a subsequent full-blood donation. Control group: 45 full-blood donors</td>
<td></td>
</tr>
<tr>
<td>Bellitti, 1994 [37]</td>
<td>Italy</td>
<td>Observational</td>
<td>20 (13M, 7F)</td>
<td>Range 25-45</td>
<td>Full blood (F: 5 ml/kg; M: 6 ml/kg)</td>
<td>Within subject comparison 1st and 4th donation</td>
</tr>
</tbody>
</table>

Continued on next page
<table>
<thead>
<tr>
<th>Author, year</th>
<th>Country</th>
<th>Study type (design)</th>
<th>Sample size (gender a)</th>
<th>Age (year, mean±sd b)</th>
<th>Donation type</th>
<th>Prior donations (mean±sd b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferguson, 1997 [55]</td>
<td>UK</td>
<td>Quasi-experimental</td>
<td>215 (75M, 137F, 3 not recorded)</td>
<td>Mean 23, range 18-57</td>
<td>Blood donation</td>
<td>4 groups; FTD, 1-2 times, 3 times, 4 or more times</td>
</tr>
<tr>
<td>Byrne, 2005 [61]</td>
<td>Canada</td>
<td>Observational</td>
<td>610 (263M, 347F)</td>
<td>22.4±7.2</td>
<td>Blood, 450 ml</td>
<td>3.6±7.3</td>
</tr>
<tr>
<td>Hanson, 2009 [57]</td>
<td>Ohio</td>
<td>RCT, 2 groups: with (n=34) and without (n=31) social support</td>
<td>65 (31M, 34F)</td>
<td>22.3±7.4, range 18-57</td>
<td>Whole blood</td>
<td>Novice, 0-2 donations</td>
</tr>
</tbody>
</table>

a M for male, F for female, as reported by paper. b If reported, unless stated otherwise. c Exp - abbreviation for experiment. d FTD - First Time Donor.
post-donation levels of anxiety [61]. Furthermore, they showed a significant association with a greater difference between pre- and post-donation systolic blood pressure and higher levels of alexithymia.

In a study assessing the influence of providing passive music, Ferguson et al. showed vigilance coping occurred when music was present, a form of distraction thought to decrease anxiety levels [55]. However, the presence of music resulted in higher pre-donation levels of anxiety-provoking appraisals (rating the environment as anxiety-provoking) and higher post-donation levels of depressing appraisals (rating the environment as depressing) in less-experienced donors (≤2 donations). In more experienced donors (≥3 donations), the results were the reverse, with these donors showing lower levels of post-donation depressing appraisals. No effect was found for levels of anxiety. In a study assessing the effects of distraction on anxiety levels, Ulrich et al. showed that distraction in the form of different television programmes had an association with stress levels [56]. Thereby, a group given low distraction (nature films on television or no television), showed lower stress reactions than a group given high distraction (urban environment on television).

**Physiological stressors**

Two physiological stressors were associated with stress reactions in a blood donation setting, namely physical symptoms and donation success [9, 57]. Physical symptoms, also called donor-related symptoms, were found to be associated with higher levels of stress reactions [9, 57]. Symptoms were assessed using the Blood Donation Reaction Inventory, both the 11-item and the 4-item versions, assessing symptoms such as dizziness and light-headedness. Other measures were pain ratings of the venipuncture or fingerprick, and nurse reports. A significant negative correlation was found between post-donation anxiety and donation success [9], which implies that donors experience more stress when their donation is unsuccessful.

**Discussion**

In this review, we identified factors associated with psychological and physiological stress reactions related to donating blood, extracted from ten studies. Psychological stress reactions, measured through questionnaires, consisted of increased pre-donation levels of anxiety, fear and arousal, which declined towards the end of the donation. Physiological stress reactions included higher pre-donation heart rate and blood pressure, as well as higher post-donation cortisol levels. Fewer prior donations and a number of psychological and physiological stressors were found to be associated with higher stress reactions. Psychological stressors associated with higher stress reactions included high-distraction television, habitual anxiety, negative expectations and extraversion. An avoidant and problem-focused coping method was associated
with lower stress reactions, and expressed more by donors who had made more prior
donations. Physiological stressors associated with a higher stress reaction included
ratings of pain and physical symptoms, e.g. dizziness and light-headedness. Except
for the number of prior donations, which was shown to be relevant in seven studies,
each stressor was specified in no more than two articles.

As an indicator of psychological stress experienced by the donor, most studies assessed
anxiety through the use of standardised questionnaires. Unfortunately, the small
number of psychological stress reactions assessed might lead to a simplification of our
knowledge, as for instance nervousness or tension was not measured. All psychological
and nearly all physiological stress levels were found to be raised during registration,
declining towards the end of the donation procedure. In addition, two studies showed
anxiety peaking shortly before or after the venipuncture [57, 58]. Both psychological
and physiological reactions imply a predominantly anticipatory stress reaction, while
the physiological component might also be related to the physical efforts made by the
donors, e.g. walking or cycling towards the donation centre. Although a number of
associations are made between stress levels and hormonal parameters in the literature
[17, 63], we included only one blood donation related study examining one of these
parameters, i.e. serum cortisol [37]. In this study by Bellitti et al., the cortisol level
prior to the fourth donation was lower than that prior to the first donation. However,
cortisol levels in the first donation decreased during the donation while they stay at
a constant level in the fourth donation. It can be speculated that the physiological
stress responses (heart rate, blood pressure and cortisol) are mediated by a primarily
physiologically driven mechanism, perhaps because of a response to hypovolaemia.
However, in regard to the cortisol response, this does not seem likely since blood
loss at a blood donation is relatively small (500 mL), and the level of cortisol was
measured shortly after the donation (30 minutes). Moreover, there was not a similar
increase in pre-donation cortisol at the fourth donation as there had been in the
first donation. With the exception of this study, cortisol has not been found to be
associated with blood donations, and the influence of the increase in cortisol on the
blood product is not yet clear. In a large systematic review, Thrall et al. showed the
effects of psychological stress and physical activity on haemorheology, coagulation,
fibrinolysis and platelet reactivity [10]. Despite our sensitive search and the high
number of hits, we were unable to find a study which assessed these parameters in
a blood bank setting. In our view, this lack of findings is interesting since donation-
induced stress might affect the final blood product.

Seven studies reported an association between an increased number of prior dona-
tions and lower stress reactions around a blood donation. This phenomenon might be
explained by donor selection, with donors who show more reaction not returning to
make subsequent donations [42]. Likewise, anxiety was shown to reduce return rates
for female donors in this review [9], and physical symptoms and donation successful-
ness were associated with increased stress reactions [9, 57]. This is also in line with
findings in the literature, in which a study by France et al. showed that donors who
react do not come back [7]. Interestingly, Bellitti et al. found a reduction in cortisol
Table 3  Factors associated with stress reactions, studied in blood donors.

<table>
<thead>
<tr>
<th>Factor studied (method)</th>
<th>Author, year</th>
<th>Psychological stress measurement</th>
<th>Physiological stress measurement</th>
<th>Details</th>
<th>Direction of association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior donations</td>
<td>Kaloupek, 1984 [59]</td>
<td>Anxiety (AT, AACL, ON)</td>
<td>PD: no groups</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kaloupek, 1985 [60]</td>
<td>Anxiety (AT, AACL, ON, OTO)</td>
<td>PD: 0 or ≥1</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Basler, 1987 [58]</td>
<td>Anxiety (STAI-X1, self-constructed stress scale)</td>
<td>TD: pre-donation stress levels only. PD: TD: 0, 1, 2. WD: ?</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Breckler, 1993 [62]</td>
<td>Anxiety (MACL)</td>
<td>PD: 0, 1-2, 3-8, ≥9</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bellitti, 1994 [37]</td>
<td>Anxiety (STAI)</td>
<td>Experience only associated to cortisol.</td>
<td>PD: 1, 4</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>Ferguson, 1997 [55]</td>
<td>Anxiety (SACL, ALE)</td>
<td>PD: 0, 1-2, 3, ≥4</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Byrne, 2005 [61]</td>
<td>Anxiety (STAI)</td>
<td>Blood pressure</td>
<td>Experience only associated to anxiety.</td>
<td>PD: no groups</td>
</tr>
<tr>
<td></td>
<td>Coping method (CS, CQ)</td>
<td>Kaloupek, 1984 [59]</td>
<td>Anxiety (AT, AACL, ON)</td>
<td>Avoidant coping</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>Kaloupek, 1984 [59]</td>
<td>Anxiety (AT, AACL, ON)</td>
<td>PD: no groups</td>
<td>Negative</td>
<td></td>
</tr>
</tbody>
</table>

Continued on next page
<table>
<thead>
<tr>
<th>Factor studied (method)</th>
<th>Author, year</th>
<th>Psychological stress measurement</th>
<th>Physiological stress measurement</th>
<th>Details</th>
<th>Direction of association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donation type</td>
<td>Basler, 1987 [58]</td>
<td>Anxiety (STAI-X1, self-constructed stress scale)</td>
<td>Heart rate, blood pressure</td>
<td>TD only</td>
<td>Negative</td>
</tr>
<tr>
<td>Character traits (STAI-X2, FPI)</td>
<td>Basler, 1987 [58]</td>
<td>Anxiety (STAI-X1, self-constructed stress scale)</td>
<td>Heart rate</td>
<td>TD: habitual anxiety, negative expectations, extraversion</td>
<td>Positive</td>
</tr>
<tr>
<td>Television</td>
<td>Ulrich, 2003 [56]</td>
<td>Fear (ZIPERS)</td>
<td>Heart rate, blood pressure</td>
<td>Nature and no television (low-distraction), all measurements</td>
<td>Negative</td>
</tr>
<tr>
<td>Music</td>
<td>Ferguson, 1997 [40]</td>
<td>Anxiety (SACL)</td>
<td>None</td>
<td>Association vigilance coping and post-donation depressing appraisals</td>
<td>≤2 donations: positive; ≥3 donations: negative</td>
</tr>
<tr>
<td>Alexithymia (TAS)</td>
<td>Byrne, 2005 [61]</td>
<td>Anxiety (STAI)</td>
<td>Blood pressure</td>
<td>Alexithymia only associated to anxiety</td>
<td>Positive</td>
</tr>
<tr>
<td>Physical symptoms (BRDI, ON)</td>
<td>Hanson, 2009 [57]</td>
<td>Anxiety (STAI)</td>
<td>None</td>
<td>Assessed BDRI-4</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Continued on next page
<table>
<thead>
<tr>
<th>Factor studied (method)</th>
<th>Author, year</th>
<th>Psychological stress measurement</th>
<th>Physiological stress measurement</th>
<th>Details</th>
<th>Direction of association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donation successfulness</td>
<td>Ditto, 2006 [9]</td>
<td>Anxiety (STAI)</td>
<td></td>
<td>Assessed BDRI, association also for VP</td>
<td>Positive</td>
</tr>
</tbody>
</table>

AT: Anxiety Thermometer; AACL: Affective Adjective Checklist; ON: Observation Nurse; PD: number of Prior Donations; OTO: Observation Trained Observer; STAI-X1 and STAI: Spielberger State Anxiety Scale, measuring situational anxiety; TD: Thrombocytapheresis Donation; WD: Whole-blood Donors; MACL: Mood Adjective Check List; SACL: Stress Arousal Check List; ALE: Appraisal of Life Events; CQ: Coping Question, to assess coping method, based on Billings & Moos; CS: Coping Scale, Cope, to assess coping, based on Billings & Moos; STAI-X2: Spielberger State Anxiety Scale, measuring habitual anxiety; FPI: Questionnaire assessing extraversion, neuroticism and masculinity; ZIPERS: Zuckerman Inventory of Personal Reactions; TAS: Toronto Alexithymia Scale; BDRI: Blood Donation Reactions Inventory; BDRI-4: Abbreviated, 4-item version of the Blood Donation Reactions Inventory; VP: VAS-scale Pain finger prick and venipuncture.
Giving blood: donor stress and hemostasis

levels from first to fourth donations, suggesting that a donation is not a stressful event in itself, and the stress exhibited might be related to the emotional component of a never-experienced-before event [37]. In conclusion, the above-mentioned finding might indicate that donors who do not experience major adverse events in the first few phlebotomies return, which, through a process of habituation, results in concomitant reduced stress levels.

A number of stressors were assessed in just two studies. While being noteworthy, their relevance and direction of association are therefore disputable. For instance, distraction in the form of television or music gave mixed results. Ferguson et al. found that the presence of music had no influence on experienced stress [55]. However, music was shown to have the opposite effects on environmental appraisals for donors who had made more than, or two or less donations, being beneficial in the former situation, and detrimental in the latter one. In contrast, Ulrich et al. showed stress reactions were lower in a group exposed to low distraction compared to those in a group exposed to high distraction [56]. Unfortunately, Ulrich et al. did not present details of the number of prior donations, which might explain the different findings. The effects of coping method on the stress reaction were described in two studies, both conducted by Kaloupek et al. [59, 60]. In their view, coping strategies are seen as “trait” measures and influence the way people react in general to stressors. They showed avoidant and problem-focused coping reduced anxiety around a blood donation. A number of character traits - alexithymia, habitual anxiety, negative expectations and extraversion - were also associated with higher stress reactions [58, 61]. Whilst the first three observations are not difficult to understand, the last one is. However, according to the authors, extraversion accounted for only a small part, and was therefore not considered relevant for practical intervention [58]. The psychology of apheresis donors as well as the physiology of apheresis donations might differ from the psychology and physiology at whole blood donation, since apheresis is more time consuming and often performed more regularly than a standard whole blood donation. This may point towards a greater commitment and more involvement from the plasma-donor, as well as an increased self-esteem, which was also shown by Veldhuizen et al. [64]. Veldhuizen et al. also showed that plasma donors had less (pre-donation) anxiety and had made significantly more prior donations than the whole blood group.

The findings in this review indicate that stress levels are increased at the start of the donation period, with inexperienced donors showing higher stress levels. Nurses and physicians dealing with donors should be aware of this phenomenon, and make efforts to comfort the donor at this point. Although people respond differently after confrontation with a stressor, such as a bruise or the insertion of a needle, low distraction television such as nature films might help donors who need to be distracted. Reducing the level of stress experienced by donors can be beneficial for donor retention.

Limitations While the strength of this study is its systematic retrieval and description of the literature, this may also be a source of weakness. Our purpose was to perform
Chapter 2. Factors for stress reactions in blood donors

A comprehensive search, including all possible factors which might be associated with stress reactions around a blood donation. Although we obtained a large number of citations, we cannot be sure that we did not miss relevant papers. We tried to deal with this problem by scanning the references of included studies, which yielded no additional studies. The comparability between the studies may also present a limitation. All studies originated in the USA or Europe. Although we have no indication that whole-blood donors were paid for donation, it is common in the USA to pay plasma donors, while this is not allowed in most European countries. Stress reactions between paid and unpaid donors might be different because of, for instance, motivational differences.

The number of studies in the field of stress reactions and stressors in blood donation is rather limited. Many issues, such as the relation between negative experience and stress reactions in future donations, or the relation between stress reactions and blood products, require further research. Based on the literature we reviewed in this study, we conclude that stress reactions appear to be present around blood donations. In particular, anxiety levels rise at the start of a donation. An increased number of prior donations is associated with a reduced level of stress reactions, while negative experiences are associated with increased stress levels. Physicians and nurses dealing with donors should be aware of this phenomenon, and make efforts to comfort the donor at this point.
Part II
Anticipatory stress and negative experiences
CHAPTER 3

Negative experiences and pre-donation blood pressure at the subsequent donation in blood donors

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Ingrid J.T. Veldhuizen
Katja van den Hurk
Wim L.A.M. de Kort
Judith K. Sluiter
Monique H.W. Frings-Dresen

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Chapter 3. Negative experiences and blood pressure

Abstract

Background: Negative donation experiences, like being deferred or experiencing an adverse reaction, might upset blood donors, resulting in anticipatory stress responses such as elevated blood pressure at the subsequent visit. We therefore explored associations between blood donors’ negative donation experiences and their blood pressure at the subsequent visit.

Study design and methods: Blood pressure of donors with no history of negative experiences in three consecutive donations was compared to the blood pressure of donors with a negative experience during the second of the three donations. Blood pressure (systolic and diastolic) measured prior to the third donation was compared between the two groups, using linear regression analyses. Two types of negative experiences (adverse reactions and deferral) were analysed, stratifying for donation type and sex, and adjusting for age and predonation blood pressure at baseline.

Results: In total 248,118 (50% female) donors were included in the analyses. Eleven percent (26,380 donors, 61% female) had experienced a negative experience. Fainting and dizziness were associated with significant \( (p<.05) \) increases in systolic blood pressure: in men, 3.0 mmHg (fainting) and 2.0 mmHg (dizziness); in women, 2.0 mmHg (fainting) and 1.4 mmHg (dizziness). Deferral was associated with significant \( (p<.05) \) increases in both systolic (men 0.7 mmHg, women 0.3 mmHg) and diastolic (men 0.2 mmHg, women 0.3 mmHg) blood pressure.

Conclusion: Whole blood donations with negative experiences were associated with a statistically significant higher predonation blood pressure at the subsequent visit. This indicates that negative experiences might cause an anticipatory stress reaction in a subsequent donation.
Giving blood: donor stress and hemostasis

Introduction

Blood donation is generally seen as a voluntary and socially beneficial activity [40]. However, sometimes donating blood results into negative consequences for donors. For example, self-reported prevalence rates of up to 10% have been shown for adverse reactions such as vasovagal reactions, needle reactions, and fatigue [5]. These experiences might upset the donor, as indicated by the association between the occurrence of adverse reactions during a blood donation and increased (post-donation) anxiety at the same donation. Also, stress reactions (e.g. anxiety) and adverse events (e.g. needle pain and vasovagal reactions) have been observed to have a clear negative effect on retention rates [7, 9, 36, 42]. Even short-term temporary deferral (for instance because of low hemoglobin, or a cold) has also been shown to cause psychological distress [9, 65]. Since adverse reactions and deferral are associated not only with short-term donor discomfort but also with lower retention rates [5–7], one can speculate about a possible negative long-term effect of adverse reactions and deferral on stress reactions.

Anxiety is considered to be a general stress reaction [14]. Stress reactions are common phenomena, and stress-inducing situations are thought to be quite diverse and to differ between individuals and circumstances [15]. In addition to a psychological stress reaction such as anxiety, several physiological stress reactions have also been described in the literature. These physiological stress reactions include increases in heart rate activity (HR) and blood pressure (BP) [32, 46], as well as changes in certain haematological parameters (e.g. FVII) [66, 67]. Also anticipatory stress, worrying about the future, has been shown to cause physiological arousal [33], such as higher blood pressure [32]. A number of studies show stress reactions peaking at the beginning of the blood donation procedure, indicating a predominantly psychological, anticipatory stress reaction [9, 55–62].

Negative experiences, both deferral and adverse events, thus seem to be able to induce a psychological stress reaction at the same donation, although this effect may be different for plasmapheresis and whole blood donors [58]. However, it is unclear whether donors who have experienced an adverse event show an increased stress response at their subsequent blood donation. Does a negative donation experience result into an anticipatory physiological stress reaction at the subsequent visit? It was the realization of this lack of knowledge that formed the basis of our research question: Do donors with and without a negative experience show different blood pressure levels at the predonation screening of the subsequent visit? Based on the literature on stress reactions to date, we expected that donors with a negative experience (either a deferral or an adverse event) would show a higher anticipatory stress response in the subsequent visit than donors who did not have a negative experience. The aim of our study was therefore to explore the associations between negative donation experiences and blood pressure at the subsequent visit in blood donors.
Figure 1 Donation history per group.
Materials and methods

Participants

Details of the donor registration and invitation, and of the donation process in the Netherlands, were described previously by Atsma et al. [30]. Briefly, donors must be between 18 and 70 years of age, and must meet certain health and lifestyle criteria before each donation. Candidate donors are seen by either a donor nurse or physician before each donation, at which point blood pressure and haemoglobin level are measured. Only donors who had attempted to donate blood at least three times in the period 2011 - 2013 and were registered as whole blood or plasmapheresis donor were included in this study. All donations are registered in our national donor management database eProgesa.

To be able to clearly distinguish a stress reaction between donors who had had a negative experience and those who had not, two groups of donors were included in the study. For all participants in both groups, data were available for three consecutive donations. A schematic overview of the donation history per group is provided in Fig. 1. In both groups, the initial donation (donation 1) needed to be without negative experiences, so that this donation could serve as a baseline for blood pressure. The second donation (donation 2) differed between the groups: donors in group 1 did not have a negative donation experience, whereas group 2 comprised all donors who did have a negative donation experience. Blood pressure is measured early in the donation procedure at the predonation screening. Therefore, the current donation (donation 3) needed to be without deferral. Since no adverse reactions are expected before the blood pressure measurement, which takes place before the actual donation, donors who did experience an adverse reaction at donation 3 (which we assume occurred after the measurement of blood pressure) were included in the analysis.

Data

Blood pressure is routinely measured during the predonation screening while the person is seated. It is measured according to standardized operating procedures, using a digital oscillometric blood pressure monitor, type Omron HEM-907 Intellisense (Omron Healthcare, Lake Forrest, IL, USA). If an irregular pulse, an SBP ≤90 or ≥180 mmHg, or a DBP ≤50 or ≥100 mmHg, is present, the donor will be deferred for donation.

In 2010, a coding system was introduced to record the occurrence of donor complications or procedural problems in eProgesa. It entails classifying donor complications into types that are compatible with the International Society for Blood Transfusion surveillance classification, which is based on clinical signs and symptoms [68]. For this study, negative experiences were categorized, in order of decreasing (donor) severity as (i) fainting, (ii) dizziness, (iii) physical problems (e.g. headache, haematoma), (iv)
### Table 1  Demographic and donation characteristics in whole blood and plasmapheresis donors, by donation negative experience and gender.

<table>
<thead>
<tr>
<th></th>
<th>Without negative experiences (Group 1)</th>
<th>With negative experiences (Group 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole blood</td>
<td>Plasmapheresis</td>
</tr>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
</tr>
<tr>
<td>Number (n)</td>
<td>89,315</td>
<td>89,148</td>
</tr>
<tr>
<td>First time donors at donation 1 (n)</td>
<td>72</td>
<td>239</td>
</tr>
<tr>
<td>Age (years)</td>
<td>48±14</td>
<td>44±14</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.8±3.2</td>
<td>25.1±3.9</td>
</tr>
<tr>
<td>Hb (mmol/L)</td>
<td>9.4±0.6</td>
<td>8.6±0.5</td>
</tr>
<tr>
<td>SBPbaseline (mmHg)</td>
<td>138±15</td>
<td>129±15</td>
</tr>
<tr>
<td>DBPbaseline (mmHg)</td>
<td>79±10</td>
<td>77±10</td>
</tr>
<tr>
<td>Number of prior donations (n)</td>
<td>34</td>
<td>14</td>
</tr>
<tr>
<td>Interval (donation -1 and 0, in days)</td>
<td>72</td>
<td>147</td>
</tr>
</tbody>
</table>

|                          | 8,223       | 14,083         | 1,962       | 2,112          |
| First time donors at donation 1 (n) | 239         | 863            | 0           | 2              |
| Age (years)              | 48±14       | 44±14          | 53±12       | 47±13          |
| BMI (kg/m²)              | 25.1±3.9    | 26.0±3.2       | 24.7±4.0    | 25.4±4.0       |
| Hb (mmol/L)              | 8.9±0.9     | 8.0±0.7        | 9.4±0.8     | 8.4±0.7        |
| SBPbaseline (mmHg)       | 138±15      | 129±15         | 127±16      | 125±16         |
| DBPbaseline (mmHg)       | 79±10       | 77±10          | 79±10       | 75±10          |
| Number of prior donations (n) | 84          | 51             | 30          | 9              |
| Interval (donation -1 and 0, in days) | 35          | 112            | 140         | 56             |

- a Reported as mean±SD.
- b Reported as median (25th–75th quartiles).
- c Significantly different between group 1 and 2, p<.05.
Giving blood: donor stress and hemostasis

other donor complications (rare complications, such as thrombosis, arterial problems, citrate reactions, allergy), (v) non-donor complications (machine malfunction, problems with the procedure or with disposables - all without donor complications), and (vi) deferrals. If a donor qualified for multiple categories, that donor was included in only the most severe category. For example, a donor experiencing dizziness and fainting was assigned to the category (i) fainting.

Statistical analysis

T-tests were used to detect differences between group 1 and group 2 (stratified for sex and type of donation). Linear regression analyses were used to examine the relationship between donors' negative experiences and subsequent blood pressure. All analyses were performed separately for men and women, and donation type (whole blood or plasmapheresis donation). Type of donation at donation 2 determined if donors were included in the whole blood or plasmapheresis donor group. Some donors switched between types of donation, for instance, because they had travelled to countries with infectious diseases, or because the donor wanted it, or the blood supply asked for it. Since switching might cause additional stress, we performed a number of additional regression analyses, separating these groups of 'changing' donors.

Baseline blood pressure (i.e. blood pressure at donation 1), age, the time interval between the second and current donations (i.e. the time interval between donation 2 and donation 3), the total number of prior donations, and body mass index (BMI) were taken into account as potential confounders and effect modifiers. Results are presented as adjusted regression coefficients ($\beta$) with 95% confidence intervals. Unless stated otherwise, significance was assumed at $p<.05$. Analyses were performed using IBM SPSS Statistics version 21.

Results

In total, 248,118 (50% female) donors were included in the analyses, of whom 200,769 (51% female) were whole blood donors, and 47,349 (42% female) plasmapheresis donors. In respect to the included donors, excluded donors (grouped by sex and type of donation, total n= 192,567) were smaller, younger and less experienced, and had a lower body weight, haemoglobin level and BMI. They also showed a lower systolic and higher diastolic blood pressure. Differences were statistically significant, yet very small and, except for age and the number of prior donations, generally below 1-3%. Demographic and donor characteristics of all donors are presented in Table 1, separately for sex, donation type and the presence of negative experiences. In total, 26,380 (11% of total, 61% female) donors encountered a negative donation experience. The independent samples t-tests revealed that, female, but not male,
### Table 2  Number of negative experiences in whole blood and plasmapheresis donors, by sex.

<table>
<thead>
<tr>
<th>Complications</th>
<th>Whole blood</th>
<th>Plasmapheresis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men a</td>
<td>Women a</td>
</tr>
<tr>
<td>Number (n)</td>
<td>8,223 (8.4)</td>
<td>14,083 (13.6)</td>
</tr>
<tr>
<td>Fainting</td>
<td>89 (0.1)</td>
<td>176 (0.2)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>255 (0.3)</td>
<td>616 (0.6)</td>
</tr>
<tr>
<td>Physical problems</td>
<td>89 (0.1)</td>
<td>240 (0.2)</td>
</tr>
<tr>
<td>Other donor complications</td>
<td>72 (0.1)</td>
<td>104 (0.1)</td>
</tr>
<tr>
<td>Non-donor complications</td>
<td>1,022 (1.0)</td>
<td>2,181 (2.1)</td>
</tr>
<tr>
<td>Deferral</td>
<td>6,696 (6.9)</td>
<td>10,766 (10.4)</td>
</tr>
</tbody>
</table>

a Data presented as number (% of total number of men or women).
Giving blood: donor stress and hemostasis

Predonation blood pressure at baseline (donation 1) was significantly higher in group 1 than in group 2 (Table 1). The low number of first time donors observed in the plasmapheresis groups reflects the procedure in the Netherlands, where donors do have to make at least one whole blood donation before being allowed to donate plasma. However, in some cases this rule is abandoned, for instance when donors have traveled to malaria-districts which otherwise would lead to long term deferral for a whole blood donation.

Negative experiences

Of the 248,118 donors included, 11% (26,380 donors) experienced a donation complication or a deferral (Table 2). In total, 19,486 (61% female) donors were deferred. Furthermore, 300 (68% female) donors fainted, 1,047 (72% female) donors experienced dizziness, 738 (63% female) donors experienced other physical problems such as haematomas, and 270 (57% female) donors experienced other donor-related complications. A total of 4,539 (61% female) donors encountered non-donor related problems, such as problems related to the procedure, machine, or disposables.

Blood pressure changes

Figures 2 and 3 show the unadjusted blood pressures for whole blood donors without a negative experience (group 1) and for the different types of negative experiences, for women (Fig. 2) and men (Fig. 3) separately. Compared to donors with uncomplicated donations, donors experiencing a vasovagal reaction (i.e. fainting or dizziness) had a lower blood pressure in all three donations. Only the results of the whole blood donors are shown, because results in the plasmapheresis group rarely reached significance. Blood pressure differences of up to 3.0 mmHg between donors without negative experiences and donors with negative experiences were observed. Amongst whole blood donors, men who fainted during the second donation had a 3.0 mmHg higher systolic blood pressure than men who did not, whereas women who fainted during the second donation had a 2.0 mmHg higher systolic blood pressure than women who did not. A significantly lower (-1.0 mmHg) diastolic blood pressure was observed in men who experienced dizziness vs. men who did not. Deferral was associated with small but significant ($p<.05$) increases of 0.3 to 0.7 mmHg for both systolic and diastolic blood pressure, in almost all groups. Although in the whole blood donor group the general tendency was that for most negative experiences a higher blood pressure was found, this finding was not significant for all forms of negative experiences. Results in the plasmapheresis donor group rarely reached statistical significance. Adjustment for the length of the interval period between the second and current donations (donations 2 and 3), or the total number of prior donations did not alter the results. A model containing age, baseline blood pressure and BMI found only a few relevant changes in regression coefficients between the original model, containing only age and baseline blood pressure. These relevant changes occurred
only in regression coefficients that indicated no significant associations in both the original and the adjusted model. Since relevant changes in regression coefficients in the original model were more conservative, thereby underestimating the effects we found, we decided not to include BMI in our model. No significant interaction effect was found for age.

**Type of donation**

When separated between plasmapheresis and whole blood donation, regression analyses showed significantly different results compared to whole group analyses: plasma donors showed almost no statistically significant associations. Additional separation between groups of 'changing' donors revealed no differences in main effects, nor in the direction of the effects. Therefore, type of donation was only separated into whole blood and plasmapheresis.

![Figure 2](image)

**Figure 2** Blood pressure and negative experiences, in female whole blood donors. The bars show the systolic (upper border) and diastolic (lower border) blood pressure in mmHg during the three donations, per type of negative experience.
Giving blood: donor stress and hemostasis

Discussion

In a large group of donors with a total of 26,380 negative experiences there was a statistically significant positive association between negative donation experiences and blood pressure at the subsequent donation for whole blood donors. Whole blood donors with a history of negative experiences generally had a higher blood pressure than donors without a history of negative experiences. The most prominent negative experiences associated with increased blood pressure were fainting and dizziness, which were associated with systolic blood pressure increases of up to 3.0 mmHg in both male and female whole blood donors. Machine problems (non-donor complications) were also associated with significant increases in systolic (men and women) and diastolic (women) blood pressure, but to a far lesser extent. In contrast to
Table 3  Associations of negative experiences at donation 2 with pre-donation blood pressure at the next visit.

<table>
<thead>
<tr>
<th>Experience</th>
<th>Whole blood a</th>
<th>Plasmapheresis a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
</tr>
<tr>
<td></td>
<td>Systolic blood pressure $\beta$</td>
<td>Diastolic blood pressure $\beta$</td>
</tr>
<tr>
<td>Fainting</td>
<td>3.0 b</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>(0.6;5.4)</td>
<td>(-1.4;1.8)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>2.0 b</td>
<td>-1.0 b</td>
</tr>
<tr>
<td></td>
<td>(0.5;3.4)</td>
<td>(-1.9 - 0.0)</td>
</tr>
<tr>
<td>Physical problems</td>
<td>-0.1</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>(-2.5;2.3)</td>
<td>(-0.4;2.7)</td>
</tr>
<tr>
<td>Other donor complications</td>
<td>2.4</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>(-0.3;5.1)</td>
<td>(-1.8;1.7)</td>
</tr>
<tr>
<td>Non-donor complications</td>
<td>0.7 b</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>(0.0;1.2)</td>
<td>(0.2;0.8)</td>
</tr>
<tr>
<td>Deferral</td>
<td>0.7 b</td>
<td>0.2 b</td>
</tr>
<tr>
<td></td>
<td>(0.4;1.0)</td>
<td>(0.1;0.4)</td>
</tr>
</tbody>
</table>

Data are presented as regression coefficient ($\beta$) including the 95% confidence intervals, adjusted for age and baseline blood pressure.

* Significance assumed at $p < .05$
Giving blood: donor stress and hemostasis

whole blood donors, plasmapheresis donors did not show significant changes in blood pressure related to negative donation experiences. Since plasmapheresis donors had performed more donations, this can be interpreted in line with the literature, which indicates lower stress levels in more experienced donors [69].

Our results in whole blood donors support our hypothesis that negative experiences during a donation induce stress reactions at the following visit, irrespective of the time between donations. Overall, our findings also add knowledge about which negative experiences are associated with elevated physiological stress levels in what type of donor. The most prominent associations are fainting and dizziness in whole blood donors. No studies thus far have investigated the long-term physiological stress effects after a negative experience during a blood donation. Evidence to date has been limited to explicit mental stress tests. For instance, increases in systolic blood pressure after a mental stress test ranged from 13 to 48 mmHg, and diastolic blood pressure increases ranged from 13 to 21 mmHg [32, 70, 71]. Although the associations we found were smaller, the studies mentioned above investigated the blood pressure changes immediately after the stressor, whereas we studied blood pressure changes after a prolonged time interval. Furthermore, mental stress tests are specifically designed to create a large stress response. It is therefore difficult to compare our results directly with the results of these other tests. In our study, only the long-term or anticipatory effect after about half a year was obtained. It is thus possible that, in line with the literature, the anticipatory response was greater immediately after the event and faded during the interval prior to the current donation [65, 72, 73].

We observed a fairly consistent effect of negative experiences on (systolic) blood pressure levels in whole blood donors. Overall, our results indicate a higher stress response in donors who had encountered a negative experience, including experiences such as deferral and non-donor problems. However, dizziness was associated with a significantly lower (-1.0 mmHg) diastolic blood pressure in male donors, in combination with a significantly higher systolic blood pressure. In general, a lower diastolic and systolic blood pressure was observed in the group with fainting or dizziness, in all three donations (Fig. 2 and Fig. 3), with a more pronounced difference for male donors. It can be speculated that donors, especially male donors, with a lower blood pressure are more prone to experiencing such a vasovagal reaction. In contrast to male donors, female donors show a significantly higher systolic as well as diastolic blood pressure dizziness. However, for both female and male donors diastolic blood pressure at donation 2 (at the time of experiencing the dizziness) is at its lowest, rising again at donation 3. Unfortunately, besides speculation we cannot explain the negative diastolic blood pressure in male donors.

A recent study of van Dongen et al. showed that women experience more adverse events during blood donation, but the negative effect of adverse events on retention was stronger in men; that is, after experiencing an adverse event, men were less likely to show up for a subsequent donation [42]. In line with this and with the findings of other studies [74, 75], we found that male donors who encountered a negative experience waited longer before making a subsequent donation. It might
well be the case that the factors influencing this prolonged interval between donations differ between donors who experience a complication and donors who are deferred. A complication might cause more anticipatory stress, while deferral perhaps raises initial stress, but its effect fades over time [65, 72, 73]. This could also account for why deferral had a smaller (though still significant) effect than complications. Although the interval between plasmapheresis donations is much smaller than between whole blood donations, virtually no statistical differences were found in this group. The lack of findings here might be due to the small groups, as well as being a more experienced donor.

We investigated a large set of data, comprising all donors who had attended three donations in 2011-2013 and had or had not encountered a negative experience in the second of these three donations. This has enabled us to be the first to investigate the associations between blood pressure and a number of negative experiences, separated for plasmapheresis and whole blood donors. Our study revealed relatively small differences in blood pressure as a result of negative experiences, sometimes with large confidence intervals. Blood pressure measurements are notoriously variable, for instance due to seasonal changes [76] and water loading [77] (water loading is a technique described in the literature for averting a vasovagal reaction: drinking water shortly before donation reduces the risk of fainting, by raising blood pressure). However, as the group of donors investigated was large, it is unlikely that random differences influenced the group means to a large extent. In relation to the clinical relevance of an increase of 3.0 mmHg, we would like to point out that - indeed - no deferral or health consequences can be based upon this value. However, we consider the increase in blood pressure merely as an indication of stress. A larger increase might thus indicate more stress, or discomfort experienced by the donor.

In our study, a low prevalence rate of negative experiences was observed. Other studies showed (self-reported) prevalence rates of up to 10% for adverse events [5, 7], but in our study the total incidence rate of complications was <4%. This is probably due to the set-up of the study: donors were included only if they had given three donations in the previous two years. In the literature, it is reported that retention is lower after an adverse event or deferral than after a uncomplicated donation [6, 7, 42]. It is therefore likely that some of the donors who encountered a negative experience in donation 1 or donation 2 did not show up for a following donation, and were thus not included in our study. Unfortunately, our set-up does not allow us to estimate the number of these 'no shows'. Furthermore, although plausible, it remains uncertain whether these donors would be 'more' stressed than donors who did return for a following donation.

Despite the interest in the different effects of deferral and adverse reactions in a blood donation setting [5–7, 31, 77, 78], physiological stress effects of deferral or other negative experiences on subsequent donations are overlooked, and psychological effects are only studied rarely. Our study is the first to indicate a difference in predonation blood pressure at the subsequent visit between whole blood donors with an earlier negative experience and donors without such an experience. After stratifi-
cation for sex and adjusting for age and baseline blood pressure, whole blood donors with a negative experience were found to have higher blood pressure than donors without negative experiences. In our view, this indicates that for these donors, negative experiences might indeed cause an anticipatory stress reaction in the subsequent visit. More research is needed to investigate whether possible interventions aimed at preventing and handling negative experiences, both adverse reactions and deferral, are justified and necessary to create a pool of relaxed and healthy donors.
CHAPTER 4

Negative experiences and pre-donation blood pressure at the subsequent donation: the role of attitude and anxiety in blood donors

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Ingrid J.T. Veldhuizen

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Abstract

Background: Negative experiences (NEs) have been shown to result in an increased stress response, as indicated by blood pressure, at the subsequent donation. This response might be influenced by how the donor rates the donation in terms of importance and pleasantness [affective attitude (AA) / cognitive attitude (CA)], or by anxiety about donating blood. We investigated the effect of AA/CA/anxiety on the impact of NEs on pre-donation blood pressure (pd-BP) in the subsequent donation.

Study design and methods: pd-BP at visit 3 was compared between donors with and without a history of NEs during or after their first two visits (visit 1: medical check, visit 2: first donation). The effect of AA/CA/anxiety (measured one month prior to visit 1 on a 7-point scale) on visit 3 pd-BP was explored using linear regression and interaction analyses. Analyses were stratified for gender, age and pd-BP at visit 1, which were taken into account as confounders.

Results: In 1106 first-time blood donors (70% female), 632 donors (57% of total) indicated an NE at their first donation. Mean scores for AA/CA/anxiety were 5.2/6.5/2.2 (men without NE), 4.8/6.3/3.0 (men with NE), 5.2/6.6/2.6 (women without NE) and 4.8/6.6/3.2 (women with NE). No significant associations were found for NE and pd-BP at visit 3, after adjusting for confounding. Out of 48 interaction effects, four were significant, but effects were small and inconsistent.

Conclusion: In donors who had had negative experiences during their first donation, anxiety and attitude to donation did not influence their pre-donation blood pressure at their subsequent visit.
Introduction

Blood donation is a voluntary activity and associated with beneficial effects such as feeling satisfied or being more alert, as well as feeling better in general [8, 79]. Unfortunately, donating blood sometimes results in adverse donation events (e.g., fainting, dizziness, bruising), which can even cause donors to withdraw permanently [4, 7]. In a recent systematic review it was shown that adverse events, unfamiliarity with donating blood, and certain ways of coping are associated with increased stress reactions such as a heightened state of anxiety [69]. Besides a positive correlation between pre-donation (‘non-acute’) fear and donor reactions [80], having had a negative donation experience has also been found to have more long-term effects, and was found to be associated with a higher pre-donation blood pressure at the subsequent visit [81]. When the pre-donation blood pressure was studied in a large cohort of healthy blood donors (N=248,118) and the pre-donation blood pressure of donors with a negative donation experience (e.g. vasovagal reaction, deferral) was compared with that of donors without such an experience, Hoogerwerf et al. found that donors with a negative experience had a raised systolic and diastolic blood pressure at the subsequent donation attempt. This is in line with literature, showing a prolonged increase in blood pressure following a mental stress test [32, 82]. Moreover, anticipatory stress, i.e. worrying about the future, has also been shown to cause raised blood pressure [32]. Hoogerwerf et al. posited that the raised blood pressure at the subsequent visit could therefore indicate increased anticipatory stress as a response to the negative experience.

Several studies have explored the effects of interventions to reduce negative experiences during a donation, for instance, reducing the number of vasovagal reactions by water loading [77], using applied muscle tension to reduce vasovagal reactions [31], or the effect of distraction on self-reported physiological reactions in first-time donors [78]. However, the presence of a psychological or physiological anticipatory stress response at the following visit as a result of this negative experience has been mostly overlooked. In addition, the influence of the donor’s attitude and anxiety towards a future donation after having experienced such an event is unclear. Attitude, both cognitive and affective, is a measure of how the donor rates the behaviour in terms of importance and pleasantness, and has been found to be a predictor of the intention to donate in the future [34]. The role of trait donation anxiety how the donor rates the donation in advance in terms of fear is still being debated: some studies show no difference in intention to donate [35], whereas others present evidence of a negative influence on the intention to donate [36].

Summarising the above, there is evidence indicating that donors with a negative donation experience might have anticipatory stress at their following donation visit, as expressed by a raised pre-donation blood pressure. The actual impact of the negative event how the donor handles and perceives the negative event might also be influenced by the donor’s attitude or anxiety. However, the relationships between the attitude and anxiety arising from previous negative donation experiences and
future donation stress responses remain unclear.

To address this lack of knowledge, we formulated the following research question: does the donor’s attitude and anxiety concerning donating blood influence the association between negative experiences and pre-donation blood pressure in the subsequent donation? We hypothesised that inclusion of the donor’s attitude or anxiety in a regression model would lead to significant interaction effects. Thus we expected donors scoring high on affective or cognitive attitude or low on anxiety would have a lower pre-donation blood pressure after a negative experience compared with donors scoring low on affective or cognitive attitude or high on anxiety.

**Methods**

**Procedure**

An overview of the donor registration and invitation procedures in the Netherlands is provided by Atsma et al. [30]. In short, potential donors (18-65 years old) initially register via e-mail and make an appointment for a medical, donor-eligibility check that involves giving a blood sample for testing, but without donating. Donors found to be eligible are sent a card inviting them to attend the blood bank to make a donation. There, candidate donors are seen by a donor physician or nurse, at which point blood pressure is measured. Donations are registered in the donor management database eProgesa.

The procedure for this study is described in detail elsewhere [42] and was approved by the Medical Advisory Committee of Sanquin. In short, all who registered as donors between August 2008 and April 2009 received a questionnaire 10 days prior to their medical check (first questionnaire, n = 4,861; response rate 64%), and 1 month after their first blood donation (second questionnaire, n=2,438, response rate 78%). The data used in this article were collected from both questionnaires and relate to donors who attended at least one donation session after their initial donation. Thus, the donors included in the study had visited the blood bank at least three times: their medical check (visit 1), first donation (or donation attempt, visit 2), and second donation (or donation attempt, visit 3). In Fig. 1, the hypothesised moderating effect of the donor’s attitude and anxiety on pre-donation blood pressure after a negative experience is shown. To achieve a detailed overview of the donor’s career, each donor was linked to his or her blood donation record (eProgesa). Data extracted were the date of donation, the number of days between donations, hemoglobin (in mmol L$^{-1}$) and blood pressure (systolic and diastolic, in mmHg).
Figure 1 Visualization of the moderating effect of the donor’s attitude and anxiety on pre-donation blood pressure at visit 3, after having a negative experience at visit two, after adjusting for age and baseline pre-donation blood pressure (visit one).
Donor attitude

The donor’s attitude and anxiety were assessed in the first questionnaire, prior to their medical check, using the approach described in detail by van Dongen et al. [42, 83]. The questionnaire collected data on the following three variables:

– affective attitude towards donating, assessed by rating three bipolar statements on a 7-point scale (giving blood regularly the coming 2 years seems to me: (1) irksome - fun, (2) unpleasant pleasant, (3) scary not scary);

– cognitive attitude towards donating, also assessed by rating three bipolar statements on a 7-point scale [giving blood regularly the coming 2 years seems to me: (1) negative - positive, (2) useless useful, (3) not worth it - worth it];

– donation-related anxiety, assessed by rating three statements on a 7-point scale ranging from 1 (not at all) to 7 (completely) [(1) I am nervous and/or tense about blood donation, (2) I am afraid of needles, (3) I’m sometimes afraid of becoming unwell or faint at a blood donation].

For each variable, a mean score was calculated, for use in the analyses.

Negative experiences

Negative experiences, i.e. adverse events and deferral, during visit 2 (first donation or donation attempt) were assessed in the second questionnaire, 1 month after the visit. Six items shown to be representative for measuring adverse physical reactions were taken from the Blood Donation Reactions Inventory [49, 84]. Items assessed were the presence of dizziness, headache, nausea, sweating, hyperventilation, and fainting. In addition, four consequences of needle insertion were measured and fatigue was assessed. With the exception of deferral, which was scored yes or no, all items were scored on a 5-point scale ranging from 1 not at all to 5 to an extreme degree. Four categories were used: (i) vasovagal symptoms (i.e. fainting, dizziness, nausea), (ii) needle reactions (i.e. bleeding, hematoma), (iii) fatigue, and (iv) deferrals. Donors replying ‘no’ for deferral or scoring a 1 for all the other items thus had no negative experiences and formed the group without negative experiences. To be able to distinguish clearly between donors with and without negative experiences, donors replying yes for deferral or scoring a 4 or 5 for any of the other items were categorised accordingly. If a donor qualified for multiple categories, inclusion was based on the most severe category. Thus a donor experiencing dizziness and a haematoma was assigned to category (i), vasovagal symptoms.

Blood pressure

Blood pressure, serving as dependent variable, was routinely measured during the pre-donation screening while the person was seated. It was measured according
to standardised operating procedures, using a digital oscillometric blood pressure monitor, type Omron HEM-907 Intellisense (Omron Healthcare, Lake Forrest, IL, USA). If the pulse was irregular or <50 or >110 beats per minute, or if SBP was ≤90 or ≥180 mmHg, or DBP was ≤50 or ≥100 mmHg, the donor was seen by a donor physician, who decided whether the donor was eligible to donate.

**Statistical analysis**

This study used a between-subjects, observational design. Donors were included when they made at least three visits and had no missing values on both the attitude and anxiety scales. In addition, only donors with a score of 1 (no negative experience), or 4 or 5 (negative experience present) were included in the analyses, resulting in a final sample of 1,106 donors for analysis. On this set of donors, an independent-samples t-test was performed to identify differences between donors with and without a negative experience. Statistical significance was set at \( p < .05 \). Linear regression analyses were performed, exploring the effect of negative experiences during visit 2 (as independent variable) on pre-donation blood pressure at visit 3 (as dependent variable): see Fig. 1. First, a crude model including the four categories of negative experiences was estimated. Second, to adjust for confounding, the model was extended with age and pre-donation blood pressure at baseline. In these models, associations between negative experiences and pre-donation blood pressure were estimated. These models give insight into the effect of a negative experience on pre-donation blood pressure at the subsequent visit. Lastly, interaction analyses were performed for affective attitude, cognitive attitude, and anxiety, which form Models 1, 2 and 3 respectively. In these models, interaction effects of negative experiences and attitude or anxiety on pre-donation blood pressure at visit 3 were estimated. These models give insight into the moderating effect of attitude and anxiety on the effect of a negative experience on pre-donation blood pressure at the subsequent visit. Analyses were stratified for gender, as it has been shown that both the number of negative experiences and their effect on return behaviour differ between men and women [5, 41].

T-tests were used to detect differences between group 1 and group 2 (stratified for sex and type of donation). Linear regression analyses were used to examine the relationship between donors’ negative experiences and subsequent blood pressure. All analyses were performed separately for men and women, and donation type (whole blood or plasmapheresis donation). Type of donation at donation 2 determined if donors were included in the whole blood or plasmapheresis donor group. Some donors switched between types of donation, for instance, because they had travelled to countries with infectious diseases, or because the donor wanted it, or the blood supply asked for it. Since switching might cause additional stress, we performed a number of additional regression analyses, separating these groups of ‘changing’ donors.

Baseline blood pressure (i.e. blood pressure at donation 1), age, the time interval between the second and current donations (i.e. the time interval between donation
### Table 1  Demographic and donation characteristics in blood donors, by donation negative experience and gender

<table>
<thead>
<tr>
<th></th>
<th>Men Without negative experiences</th>
<th>Men With negative experiences</th>
<th>Women Without negative experiences</th>
<th>Women With negative experiences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (n)</td>
<td>205</td>
<td>128</td>
<td>269</td>
<td>504</td>
</tr>
<tr>
<td>Age (years)</td>
<td>41±12 e</td>
<td>32±11</td>
<td>39±13 e</td>
<td>31±12</td>
</tr>
<tr>
<td>Hb (mmol/L)</td>
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<td>9.3±0.6</td>
<td>8.5±0.6 e</td>
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<tr>
<td>SBP&lt;sub&gt;visit 1&lt;/sub&gt; (mmHg)</td>
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<td>131±14</td>
<td>126±15 e</td>
<td>123±14</td>
</tr>
<tr>
<td>DBP&lt;sub&gt;visit 1&lt;/sub&gt; (mmHg)</td>
<td>82±10 e</td>
<td>78±8</td>
<td>79±9 e</td>
<td>76±9</td>
</tr>
<tr>
<td>SBP&lt;sub&gt;visit 2&lt;/sub&gt; (mmHg)</td>
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<td>131±13</td>
<td>125±14</td>
<td>123±14</td>
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<tr>
<td>DBP&lt;sub&gt;visit 2&lt;/sub&gt; (mmHg)</td>
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<td>78±9</td>
<td>78±9 e</td>
<td>76±9</td>
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<tr>
<td>SBP&lt;sub&gt;visit 3&lt;/sub&gt; (mmHg)</td>
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<td>131±13</td>
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<td>122±14</td>
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<td>DBP&lt;sub&gt;visit 3&lt;/sub&gt; (mmHg)</td>
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<td>76±9</td>
<td>77±9 e</td>
<td>75±10</td>
</tr>
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<td>Interval (visit 2 and 3, in days)</td>
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<td>78 (66;108)</td>
<td>113 (107;140)</td>
<td>119 (111;157)</td>
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<td>16 (5)</td>
<td>67 (9)</td>
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* Reported as mean±SD | ** Reported as median (25th;75th quartiles) | ^ Assessed on a 1 (not) 7 (maximum) scale | † Data presented as number (% of total number of men or women) | ‡ Significantly different between donors with or without negative experiences, p<.05
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2 and donation 3), the total number of prior donations, and body mass index (BMI) were taken into account as potential confounders and effect modifiers. Results are presented as adjusted regression coefficients (β) with 95% confidence intervals. Unless stated otherwise, significance was assumed at p<.05. Analyses were performed using IBM SPSS Statistics version 21.

Results

In total, 1,106 donors were included in the analyses, predominantly women (n=773, 70%). In this group, 128 men (38% of all men) and 504 women (65% of all women) had had a negative experience during their second visit. Demographic and donor characteristics are presented in Table 1, separately for the presence of negative experiences and for men and women. Donors who had a negative experience were significantly younger than donors who did not have a negative experience. In addition, in men having a negative experience, both systolic and diastolic blood pressure were significantly lower across all three donations than in men without negative experiences. For women having a negative experience, systolic blood pressure at baseline was significantly lower and diastolic blood pressure was significantly lower for all three donations, compared with women without negative experiences. Note however, that no correction for age was applied in this t-test.

Donor attitude

Mean scores and standard deviations of the donor’s attitude and anxiety towards donation, as measured prior to visit 1 are shown in Table 1. Affective attitude was significantly higher in donors without negative experiences than in donors with negative experiences, for both men and women (5.2 vs 4.8 for both men and women). Men without negative experiences also showed a significantly higher cognitive attitude than men with negative experiences (6.5 vs 6.3). Anxiety was significantly lower in donors without negative experiences than in donors with negative experiences, for both men and women (2.2 vs 3.0 for men, 2.6 vs 3.2 for women).

Effects of attitude and anxiety on blood pressure

Associations of negative experiences at visit 2 with pre-donation blood pressure at the next visit (visit 3) are presented in Tables 2 (men) and 3 (women). In Tables 4 (men) and 5 (women), interaction effects are presented between negative experiences, e.g., vasovagal reactions, and the donor’s attitude and anxiety, e.g., affective attitude.

In short, after correction for confounding factors (age and blood pressure at visit 1), and in contrast to our earlier findings (Hoogerwerf et al. 2015), no significant associations remained between negative experiences (as independent variable) and
<table>
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<tr>
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<th>Model 1 d</th>
<th>Model 2 e</th>
<th>Model 3 f</th>
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<td>Systolic</td>
<td>Diastolic</td>
<td>Systolic</td>
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</tr>
</tbody>
</table>

Data are presented as regression coefficient (β) including the 95% confidence intervals. b Crude model, no corrections. c Crude model corrected for baseline + age. d Adjusted model corrected for affective attitude. e Adjusted model corrected for cognitive attitude. f Adjusted model corrected for anxiety.
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blood pressure (as dependent variable). Moreover, only 4 out of 48 interaction effects were found to be significant and their effects were small, inconsistent, and differed between men and women. A more detailed description of these interaction effects is given in the next section. Overall, our findings indicate no effect of the donor’s attitude or anxiety on the association between negative experiences and blood pressure.

Detailed description of the models

The following paragraphs will provide a more detailed description and explanation of the associations and interaction effects. In the crude model, associations were estimated for all four categories of negative experiences, without controlling for confounders. In men, significant associations were found for systolic blood pressure and vasovagal reactions, and for diastolic blood pressure and needle reactions (Table 2). In women, significant associations were found for systolic blood pressure and needle reactions, and for diastolic blood pressure and vasovagal reactions. In the adjusted model, in which age and baseline blood pressure (pre-donation blood pressure at visit 1) were included to adjust for confounding, none of the negative experiences reached statistical significance.

Associations for affective attitude were estimated in Model 1. Although no significant associations between negative experiences and pre-donation blood pressure occurred (Tables 2 and 3, Model 1), interaction analyses revealed a significant interaction effect for diastolic blood pressure and deferral in men (Table 4, Model 1), and systolic blood pressure and fatigue in women (Table 5, Model 1). This indicates an influence of affective attitude on pre-donation blood pressure after having a negative experience. When comparing men without and with a deferral, deferred men were found to have a baseline category effect size of -19.5 mmHg and an increase of 4.0 mmHg per point increase in affective attitude. This would imply that men scoring high on affective attitude show a higher pre-donation diastolic blood pressure after deferral whereas men scoring low on affective attitude have a lower pre-donation diastolic blood pressure. Similarly, when comparing women without and with fatigue, fatigued women were found to have a baseline category effect size of 18.5 mmHg and a decrease of 3.9 mmHg per point increase in affective attitude. This would imply that women scoring high on affective attitude showed a lower pre-donation systolic blood pressure after fatigue, whereas women scoring low on affective attitude had a higher pre-donation systolic blood pressure.

Associations for cognitive attitude were estimated in Model 2. Although no significant associations between negative experiences and pre-donation blood pressure occurred (Tables 2 and 3, Model 2), interaction analyses revealed a significant interaction effect for systolic blood pressure and needle reactions in women (Table 5, Model 2). The effect found would imply that women scoring high on cognitive attitude showed a lower pre-donation systolic blood pressure after a needle reaction, whereas women scoring low on cognitive attitude had a higher pre-donation systolic blood pressure.
<table>
<thead>
<tr>
<th>Experience</th>
<th>Crude model b</th>
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<th>Model 2 e</th>
<th>Model 3 f</th>
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<td>Diastolic</td>
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<td>(-4.7;1.5)</td>
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</table>

a Data are presented as regression coefficient ($\beta$) including the 95% confidence intervals. b Crude model, no corrections. c Crude model corrected for baseline + age. d Adjusted model corrected for affective attitude. e Adjusted model corrected for cognitive attitude. f Adjusted model corrected for anxiety.
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Finally, associations for anxiety were estimated in Model 3. Again, although no significant associations between negative experiences and pre-donation blood pressure occurred (Tables 2 and 3), interaction analyses revealed a significant interaction effect for diastolic blood pressure and deferral in men (Table 4, Model 3). The effect found would imply that men scoring high on anxiety showed a lower pre-donation diastolic blood pressure after a deferral, whereas men scoring low on anxiety had a higher pre-donation diastolic blood pressure. Summarising the above, no significant associations between negative experiences and pre-donation blood pressure at the subsequent visit were found after adjusting for confounding. Moreover, only a small number of significant interaction effects were found, with small and inconsistent effects. Significant interaction effects for affective attitude were found for: diastolic blood pressure and deferral (men, positive effect); systolic blood pressure and fatigue (women, negative effect). Significant interaction effects for cognitive attitude were found for; systolic blood pressure and needle reactions (women, negative effect). Lastly, significant interaction effects for anxiety were found for: diastolic blood pressure and deferral (men, negative effect).

Discussion

In contrast to our hypothesis, no effect of donor attitude or anxiety on the association between negative experiences and blood pressure was found. Specifically, only 4 out of 48 interaction effects between the association of negative experiences and pre-donation blood pressure on the one hand, and attitude or anxiety on the other were found significant. Moreover, despite a sample size of 1,106 donors, the observed significant effects were small, inconsistent (both in favour as well as in contrast with our hypothesis), and different between men and women. A negative donation experience was not associated with pre-donation blood pressure changes at the subsequent blood donation in this group of first-time donors. This finding adds knowledge to our findings from earlier studies. Previously, in a sample of 248,118 healthy blood donors, 99.4% of whom were experienced donors, we found a number of small, yet significant and robust, positive effects for negative experiences on blood pressure at the subsequent visit [81]. Besides a smaller sample size, this disparity in research outcomes may be mainly caused by (i) differences between new and experienced donors, and (ii) the different methods used to assess negative experiences. Still, it remains remarkable that first-time donors do not show an increased stress-response following a negative donation experience. With regards to (i) and in response to our previous findings [81]: experienced donors might be prone to a cumulative effect of negative experiences, in which the occurrence of multiple negative donation experiences or donations magnifies the stress response. Also, experienced donors with multiple perfect donations might be more surprised by the occurrence of a negative event and therefore be more stressed at a subsequent donation. Furthermore, novice blood donors, like those in the current study, might
Table 4  Interaction effects of negative experiences and attitude or anxiety on pre-donation blood pressure at visit 3 (donation #2), for men a.

<table>
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<tr>
<th></th>
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<td>Diastolic</td>
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</tbody>
</table>

a Data are presented as regression coefficient (β) including the 95% confidence intervals.
already be slightly stressed, which might mask the stress that accompanies a negative
donation experience. Thus, it is possible that novice blood donors are more upset
in the first place [80], intuitively causing a higher baseline - which will be discussed
later on. Or, on the contrary, novice donors might take a negative experience more
for granted because they think it is normal. With respect to (ii), the method used to
assess negative experiences, the current study used self-reported negative experiences
by sending donors a questionnaire 1 month after their donation. In contrast, in our
earlier study we used the regular blood bank donation records in which donor nurses
report the occurrence of negative on-site donation events. In the current study, we
found a high prevalence of self-reported negative experiences among novice blood
donors, with up to 30% of the women and 20% of the men experiencing vasovagal
symptoms. This prevalence is higher than observed in our earlier study, where the
occurrence of adverse reactions was typically below 3% and that of deferral was below
11% [81]. Self-reported incidence rates were generally found to be higher than those
recorded on-site, which is in line with other studies [8, 41]. One might expect that
only the severe negative experiences would be reported on-site, whereas self-reporting
would also include the less severe incidents and incidents occurring off-site, as well
as a potential report-bias due to selective reporting by respondents who had had a
negative experience. Also, in the questionnaire, the wording of the item asking about
the negative experience translates as 'to what extent were you affected by ' instead
of asking about the actual occurrence of the negative event. In other words, even
in the absence of severe visible physical symptoms (reported by the donor nurse),
donors perceive a negative experience as severe (using self-reporting). A comparison
between those two ways of reporting seems therefore interesting and may be partially
responsible for differences between the current study and our previous results, but is
beyond the scope of this paper.

A potential drawback of the study was the way stress responses were assessed. In
the current study, we only measured blood pressure. Blood pressure responses are
just one part of the complex cascade of stress responses. Other stress responses
are for instance increased psychological arousal and hormonal changes (e.g. salivary
cortisol). Still, blood pressure is an objective measure and is (in the Netherlands)
routinely assessed before every donation. Because blood pressure is repeatedly as-
essed in this standardised situation, and as such, is a given activity for the donors,
the influence of other factors on blood pressure responses in this particular setting are
negligible. By using corrections (age and baseline blood pressure), we made efforts
to be as conscientious as possible. Also, the assessment of blood pressure in com-
bination with the inclusion of the study participants might be a potential drawback.
The correction method of using baseline blood pressure corrects for inter-individual
changes. For instance, the generally lower blood pressure (as well as age, which was
also corrected for as younger donors are more likely to have a negative experience)
in the group of donors with a negative experience is thus corrected through this
method. However, the only baseline blood pressure available in this study was that
of the medical check: the first visit of the donor to the blood bank. The represen-
tativeness of this blood pressure could be questioned, because the first visit may in
Table 5  Interaction effects of negative experiences and attitude or anxiety on pre-donation blood pressure at visit 3 (donation #2), for women \(^a\).

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Systolic</td>
<td>Diastolic</td>
<td>Systolic</td>
</tr>
<tr>
<td>Vasovagal</td>
<td>5.4 (-3.8;14.5)</td>
<td>-0.6 (-6.9;5.8)</td>
<td>-17.1 (-39.4;5.2)</td>
</tr>
<tr>
<td>Needle reactions</td>
<td>1.0 (-10.5;12.5)</td>
<td>0.9 (-7.0;8.9)</td>
<td>-33.8 (-62.4;5.3)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>18.5 (2.3;34.8)</td>
<td>3.4 (-7.9;14.6)</td>
<td>2.9 (-22.5;28.3)</td>
</tr>
<tr>
<td>Deferral</td>
<td>6.2 (-7.8;20.1)</td>
<td>-2.3 (-12.0;7.4)</td>
<td>-10.7 (-43.9;22.5)</td>
</tr>
<tr>
<td>Interaction vasovagal</td>
<td>-0.8 (-2.7;1.0)</td>
<td>0.2 (-1.0;1.5)</td>
<td>2.7 (-0.7;6.1)</td>
</tr>
<tr>
<td>Interaction needle reactions</td>
<td>-0.4 (-2.7;1.8)</td>
<td>-0.1 (-1.7;1.5)</td>
<td>4.9 (0.6;9.2)</td>
</tr>
<tr>
<td>Interaction fatigue</td>
<td>-3.9 (-7.1;0.6)</td>
<td>-0.7 (-2.9;1.6)</td>
<td>-0.6 (-4.5;3.3)</td>
</tr>
<tr>
<td>Interaction deferral</td>
<td>-1.5 (-4.2;1.2)</td>
<td>0.4 (-1.5;2.2)</td>
<td>1.4 (-3.6;6.4)</td>
</tr>
</tbody>
</table>

\(^a\) Data are presented as regression coefficient (\(\beta\)) including the 95% confidence intervals.
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itself be stressful. Furthermore, it has been shown that stress experienced during a donation diminishes as the number of donations increases [69]. However, Bellitti et al. showed in their study that although stress reactions such as anxiety did indeed diminish with an increasing number of donations, this effect was not found for blood pressure [37]. The participants in that research had visited the blood bank three times. Other published studies have reported that retention is lower after a negative experience than after an uneventful donation [5, 7, 42]. Unfortunately, the design of the current study does not allow us to estimate the exact number of donors who did not show up after a negative experience during visits 1 or 2. It therefore remains unknown whether such donors would be more stressed than those who did return for a second or third donation. Another possible point of concern is that contrary to what we expected based on the literature, cognitive and affective attitude and anxiety were not effect modifiers but confounders. However, post hoc analyses revealed no relevant changes in associations when cognitive or affective attitude and anxiety were included in the models, which indicates that they should not be considered confounders. A post hoc correlation analysis revealed a strong and significant correlation between anxiety and affective attitude on the one hand, and vasovagal reactions on the other. Fear has been reported as important predictor for vasovagal reactions [80, 85]. It should however be noted that France et al. used on-site fear (‘acute’ effects), whereas we used anxiety levels 1 month prior to donation (‘non-acute’ effects). However, as already reported, no significant associations with the dependent variable or related interactions were observed in Model 1 (for affective attitude) and Model 3 (for anxiety).

In conclusion, we found no evidence that after a previous negative experience, a donor’s anxiety or attitude towards donation influenced the stress response in first-time donors, as measured by pre-donation blood pressure. Moreover, our results indicate that first-time donors do not show an increased pre-donation blood pressure following a negative experience, whereas previous research shows that experienced donors do show such a response. However, both findings should be interpreted with caution and should be replicated for verification. In future studies, it would be interesting to incorporate multiple stress measures, such as cortisol and psychological stress measures as well as more measurement moments to assess a potential donation induced stress response, in order to minimise negative donation experiences by targeting specific interventions.
Part III
Stress during a donation
CHAPTER 5

Psychological and hormonal stress reactions during a blood donation

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Accepted
Chapter 5. Psychological and hormonal stress when donating blood

Abstract

**Background:** Donating blood has been associated with increased stress responses, with scarce evidence indicating that levels of psychological and hormonal stress are higher pre-donation than post-donation. We investigated whether a blood donation induces psychological and/or hormonal stress during the course of a blood donation, and whether responses differed between men and women, first-time and experienced donors, and donors with high or low non-acute stress.

**Study design and methods:** In 363 donors, psychological (donation-stress and arousal) and hormonal (cortisol) stress were measured by questionnaire and salivary sample at seven key moments during a routine donation. Non-acute stress was assessed by a questionnaire. Repeated measurement analyses were performed, using the last measurement (leaving the donation center) as reference value.

**Results:** Levels of donation-stress, arousal and cortisol were significantly higher during donation than when leaving the donation center. When compared with men, women reported higher levels of donation-stress and cortisol in the first part of the visit. When compared with first-time donors, experienced donors reported lower levels of donation-stress during the first part of the visit, and higher levels of arousal but less reactivity throughout the visit. When compared to donors high on non-acute stress, donors low on non-acute stress reported lower levels of donation-stress during the first part of the visit, and showed less cortisol reactivity throughout the visit.

**Conclusion:** Donating blood influences psychological and hormonal stress response patterns. The response patterns differ between women and men, first-time and experienced donors, and between donors high and low on non-acute stress.
Introduction

Blood donation has been associated with both positive [79] and negative donation aspects [4, 7, 64] for the donor. As people differ in the way they perceive a situation as stressful, the blood donation process may also impact donors differently. Indeed, a number of studies summarized by Hoogerwerf et al. in a recent review indicate that a routine whole-blood donation is associated with psychological and hormonal stress [69]. We showed that pre-donation levels of psychological stress are reported to be higher than post-donation stress levels, and pre-donation cortisol levels in first-time donors are reported to be higher than post-donation levels. Taken together, it seems likely that a routine blood donation induces stress responses in healthy blood donors.

Common stress reactions described in the literature include multiple psychological stress reactions, such as increased levels of arousal, anxiety, fear or tension [14, 55], as well as hormonal stress reactions, such as increased cortisol excretion [17]. It is important to note that only moderate overlap between anxiety and stress has been found (at around .5 [86, 87]). In a response to acute stress, activation of the hypothalamic-pituitary-adrenal axis results in temporary elevations of circulating cortisol, superimposed on the normal circadian rhythm. Thereby, cortisol reactivity is defined as an increase of at least 2.5 nmol/L above the baseline [88]. A factor that is known to enhance a stress response is unfamiliarity with a situation [16]. In line with this, as the number of previous donations increases, the levels of psychological and hormonal stress have been shown to decrease, as expressed by lower levels of anxiety and cortisol [37, 55–62, 89]. Other factors known to influence psychological or hormonal stress reactions are gender and levels of non-acute stress. Depending on the type of challenge and stress reaction assessed, inconsistent effects are reported for gender [24–26, 57]. Non-acute stress comprises the wide range of daily hassles, or minor daily pressures, that may lead to the experience of stress. Thereby, higher levels of acute stress when driving a car in heavy or light traffic have been reported in subjects with high levels of non-acute stress [27]. Altogether, a number of factors, including the number of previous donations, gender, and levels of non-acute stress, potentially influence a stress response during a donation.

Most studies assessing stress and blood donation have measured stress, but predominantly anxiety only, at two moments: before and after the donation [9, 37, 55, 56, 59–61] Although a more detailed analysis of a blood donation procedure has been performed by three other studies, they also included only psychological anxiety measures. Of these studies, Hanson et al. assessed anxiety at three moments around the donation, and found anxiety levels were higher during the actual donation than before or after it [57]. Using also three measurement moments, Breckler et al. found high levels of anxiety up to needle insertion, and a steep decrease hereafter [62]. Finally, Basler et al. assessed anxiety at six moments around a donation, and showed stress levels were highest immediately before venipuncture [58]. This limited body of knowledge makes it difficult to assess the stress response in more detail, and to make a systematic comparison between the various moments in a donation procedure. Sum-
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marizing, to date, the various key moments in the donation procedure, e.g. arrival, medical check, needle insertion and uncoupling, and arriving at the donor canteen, have never been systematically assessed using different stress measures.

We set up a study to investigate stress induced by blood donation at multiple key moments, combining psychological and hormonal measurements. We addressed the following research question: does a blood donation induce psychological and/or hormonal stress in whole-blood donors, and are there differences between men and women, first-time and experienced donors, and donors with high or low non-acute stress? Based on the literature, we formulated four hypotheses: (1) a blood donation induces a stress response, in which stress peaks around needle insertion, (2) women and men show a different stress response during a blood donation, (3) first-time donors experience more stress than experienced donors, and (4) donors with high levels of non-acute stress exhibit higher levels of acute stress than donors with lower levels of non-acute stress. By combining multiple stress measures and key moments during a donation, we aimed to gain insights into the stress response patterns throughout a whole-blood donation procedure, potentially useful for devising and modifying interventions to minimize negative donation aspects.

Materials and methods

Participants and informed consent

The current study is the first part of a larger study investigating donation-induced stress responses and their effect on donor hemostasis (DISTRESS). A random sample of first-time and experienced male and female donors was invited by letter to participate in the DISTRESS study. Three inclusion criteria were formulated: no use of corticosteroids such as asthma medication, as they interfere with the measurement of cortisol in saliva; no use of NSAIDS such as aspirin in the four days prior to donation, as they affect platelet functioning; no use of beta blockers such as atenolol, as they affect blood pressure. Figure 1 provides a flow chart of the inclusion of the participants.

The DISTRESS-study was approved by the Medical Ethical Committee of the Academic Medical Center in Amsterdam and the Ethical Advisory Committee of Sanquin, and conducted in accordance with the principles of the Declaration of Helsinki (64th WMA General Assembly, version October 2013, Fortaleza Brazil). Reporting was done according to the Strobe Statement (version 4, October 2007).

Study procedure

On a response card or via e-mail, donors could indicate willingness to participate. If they did not respond to the initial invitation letter, one reminder was sent after four
weeks and an attempt was made to contact the donor by phone. Subjects willing to participate were phoned, to arrange a donation. During the pre-donation talk with the researcher, the donor had the opportunity to ask questions about the study, after which informed consent was obtained.

![Flow chart of participant inclusion.](image)

**Figure 1** Flow chart of participant inclusion.

**Routine donation procedure**

A routine blood donation procedure in the Netherlands starts with the donor reporting at the registration desk. Here, the donor’s identity is checked and the donor receives a donation health questionnaire (DHQ). The donor then usually has to wait several minutes before a health screening for eligibility is performed by a donor nurse or physician. This entails measuring capillary hemoglobin level with a photometer.
Figure 2 Set-up of the study procedure. Key moments of a routine donation are shown above the horizontal arrow, whereas additional measurements for the current study are below the horizontal arrow.
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(HemoCue Hb 201+, Angelholm, Sweden), measuring heart rate and blood pressure (Omron HEM-907XL, Lake Forest, USA), and evaluating the completed DHQ. If found eligible to donate, the donor is directed to the donation area. Here, the donor must sometimes wait several minutes until a bed or staff member is available. The donor sits and a nurse performs the routine venipuncture, after which blood collection starts. The blood collection process usually takes about 8-13 minutes for whole-blood donors, in semi-recumbent position. The donor is then directed to the donor canteen, where she/he can have a drink and something to eat before leaving the donation center.

Study protocol

After obtaining informed consent, the first visual analog scale (VAS) questionnaire assessing stress and arousal was completed and a non-invasive saliva cortisol sample was taken (measurement one). Next, height and weight were measured. Then the routine blood donation procedure started, with additional measurements (VAS questionnaire, cortisol) on the following key moments: reporting at the registration desk (measurement two), immediately before the screening (measurement three), at needle insertion (measurement four), and immediately after removal of the needle (measurement five). Upon arrival in the donor canteen, the donor was requested to also complete a questionnaire assessing non-acute stress (measurement six). Hereafter, the donor reported to the researcher (measurement seven). Figure 2 gives a detailed overview.

Study variables

Psychological stress

Psychological levels of stress were measured during the donation procedure using a hardcopy VAS assessing donation-stress and arousal. The scales were a vertical bar of 120 mm, with anchors at 10 and 110 mm labeled 'not stressed/aroused' and 'very stressed/aroused', respectively. Subjects were instructed to answer the two questions (VAS stress: How stressed are you right now?; VAS arousal: How aroused are you right now?) by drawing a horizontal line across the vertical line between the two anchors. A definition and sample of the assessed variable was provided. Distance to the lowest anchor was measured in mm, which resulted in a score between 0 (not stressed/aroused) and 100 (very stressed/aroused).

Hormonal stress

Cortisol concentrations (nmol/L) were obtained by taking non-invasive saliva cortisol samples, using Salivettes (Sarstedt, Etten-Leur, The Netherlands). Subjects were
asked to gently chew on a Salivette for approximately one minute. Samples were stored at Sanquin at -80 °C until sending.

Non-acute stress

To obtain a comprehensive impression of non-acute stress, three forms of non-acute stress were assessed using a digital questionnaire: distress, work-related stress, and stress from private life. Donors’ distress was assessed using the Distress Screener, a Dimension from the 4-DKL [86], which is a reproducible and valid measure. Donors had to rate three statements (During the past week, did you suffer from worry?; During the past week, did you suffer from listlessness?; During the past week, did you feel more tense?) on a three options response-scale (No (0); Sometimes (1); Regularly or more often (2)). A total score, defined as Distress, was obtained by summing the three items.

Work-related stress and stress from private life were assessed by rating two self-constructed statements (How often have you suffered stress in the past month because of work; How often have you suffered stress in the past month in private life?), on a five options response-scale (Never/almost never (0); Sometimes (1); Regularly (2); Often (3); Almost always/Always (4)).

Demographics

Date of birth (to calculate age) and lifetime number of donations were obtained from the blood bank databank (eProgesa, MAK system, France). For weight and height measurements, subjects were requested to remove shoes, heavy clothing (e.g., jackets) and to empty their pockets. Weight was measured using a digital balance (Seca 888, Hamburg, Germany). Height was measured using a wall-mounted stature meter (Stanley Microtoise 04-116, Besançon, France). Body Mass Index (BMI) was calculated from height and weight (BMI=weight/height²).

Analyses

Donors were included in the analyses if they had no missing values for the study variables. Deferred donors (e.g., because of a low hemoglobin level) were excluded from analyses.

Cortisol analysis

Analyses were performed by Technische Universität Dresden, Germany. The first batch (samples from 114 donors) was sent in April 2015. A second batch (samples from the remaining 285 donors) was sent in May 2016. In Dresden, all saliva samples
were frozen and stored at -20 °C until analysis. After thawing, Salivettes were centrifuged at 3,000 rpm for 5 minutes, resulting in a clear supernatant of low viscosity. Salivary concentrations were measured using commercially available chemiluminescence immunoassay with high sensitivity (IBL International, Hamburg, Germany). Sample and reagent handling was semi-automated, using a liquid handling robot (Genesis, Tecan, Switzerland) and quality control samples of low, medium, and high cortisol concentrations were run on each microtiter plate assayed. The intra- and interassay coefficients for cortisol were both below 8%.

Levels of salivary cortisol start increasing directly after onset of the stressor. Peak response has been reported to vary from 9 minutes [90], to 21-40 minutes after onset of the stressor or 0-20 minutes post-stressor [91], also depending on severity of the stressor [63], or subject reactivity [92]. To facilitate interpretation, we calculated times between consecutive moments and re-interpreted the actual moments of the cortisol samples. Thus, samples obtained on arrival at the donation center provide information from shortly (9-40 minutes) before arrival. Samples obtained when reporting at the registration desk provide information around arrival (average interval: 13 minutes). Samples obtained at needle insertion provide information around the screening (average interval: 16 minutes). Samples obtained at uncoupling of the needle provide information for shortly before needle insertion (average interval: 10 minutes). And finally, samples obtained when leaving the donation center provide information on sitting in the donor canteen (average interval: 19 minutes).

Non-acute stress

A combination score was constructed, with donors scoring below or on the median for all three forms of non-acute stress, i.e. distress, stress from work and stress from private life, assigned to the group with low non-acute stress. Donors scoring above the median for one or more of the three forms of non-acute stress formed the group with high non-acute stress.

Statistical analyses

Psychological and hormonal stress responses were analyzed using a repeated measurement model. As post-donation levels of stress have been reported as lower than pre-donation levels of stress [9, 37, 55–62], measurement moment seven was used as reference moment. Difference scores were calculated for the dependent variables (stress/arousal/cortisol [1-6] minus stress/arousal/cortisol [7]) on which analyses were performed. Analyses were executed with the dependent variables as within-subject variables, and, if applicable, group as between-subjects variable (i.e., gender, donation experience, non-acute stress). The effect of time on the difference scores was examined by transforming the scores into linear and quadratic trend contrast scores. The effect of group (gender, donation experience, non-acute stress) was investigated by the interaction effect. Significant effects of group on the linear and
### Table 1  Donor characteristics and outcomes for descriptive variables.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Gender</th>
<th>Donation experience</th>
<th>Non–acute stress</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Men</td>
<td>Women</td>
<td>First–time Experienced</td>
</tr>
<tr>
<td>Number</td>
<td>363</td>
<td>183</td>
<td>180</td>
<td>176</td>
</tr>
<tr>
<td>Age (years)</td>
<td>40±16</td>
<td>43±16</td>
<td>37±14</td>
<td>28±9</td>
</tr>
<tr>
<td>Previous donations (n)</td>
<td>6 (0–38)</td>
<td>13 (0–53)</td>
<td>0 (0–29)</td>
<td>0 (0–0)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.6±3.9</td>
<td>24.6±3.2</td>
<td>24.5±4.4</td>
<td>23.9±3.8</td>
</tr>
<tr>
<td>Donation stress at measure</td>
<td>16.2±16.5</td>
<td>16.1±15.2</td>
<td>16.3±17.8</td>
<td>16.5±14.5</td>
</tr>
<tr>
<td>Arousal at measure</td>
<td>67.5±25.3</td>
<td>67.3±25.2</td>
<td>67.6±25.5</td>
<td>59.7±26.1</td>
</tr>
<tr>
<td>Cortisol at measure</td>
<td>5.95±6.81</td>
<td>3.04±7.23</td>
<td>5.85±6.38</td>
<td>7.36±8.87</td>
</tr>
<tr>
<td>Distress, past 7 days</td>
<td>1 (0–2)</td>
<td>1 (0–2)</td>
<td>2 (1–3)</td>
<td>2 (1–3)</td>
</tr>
<tr>
<td>Work–related stress, past month</td>
<td>1 (1–2)</td>
<td>1 (1–2)</td>
<td>1 (1–2)</td>
<td>1 (1–2)</td>
</tr>
<tr>
<td>Stress private life, past month</td>
<td>1 (1–1)</td>
<td>1 (0–1)</td>
<td>1 (1–2)</td>
<td>1 (1–2)</td>
</tr>
</tbody>
</table>

*Presented as mean±SD or median (25th–75th percentile). |  

| b 0–100 point scale. |  

| c 0–6 point scale. |  

| d 0–4 point scale. |  


quadratic trends across time were investigated further by means of repeated effect tests, to test trend components between groups.

Results

In total, 363 whole-blood donors were included in the analyses (Figure 1). Less than 5% (n=9) had to be excluded from analyses because of random missing values, hence imputation was not applied. Donor and donation characteristics are presented in Table 1.

Stress response patterns during a donation

For donation-stress, the analyses (Table 2) revealed a significant effect of time ($F(5,358)=66.510$, $p<.001$). As illustrated by Figure 3A, levels of donation-stress in the first part of the visit were elevated, peaking at needle insertion, and decreased afterwards.

For arousal, results of the analyses (Table 2) showed a significant effect of time ($F(5,358)=11.429$, $p<.001$). Figure 3A shows a predominantly stable score for arousal, slightly elevated at the first part of the visit and increasing slightly at needle insertion.

For cortisol, a log-transformation was applied because of skewness toward lower values. Results of the analyses (Table 2) showed a significant effect of time ($F(3,356)=6.660$, $p<.001$). Figure 3A shows the untransformed values to ease interpretation: levels of cortisol show an overall decrease during the visit, indicating a decrease in stress.

Subgroup analyses

Table 3 presents results for the analyses of sub-groups (gender, donation experience, and non-acute stress).

Gender differences

For donation-stress, a significant interaction effect was found for time and gender ($F(5,357)=2.629$, $p=.024$). A repeated effect test indicated interaction occurred at needle uncoupling: before this, women scored overall higher than men; after this, no gender differences were apparent (Figure 3B).

For arousal, no significant interaction effect was found ($F(5,357)=0.893$, $p=.486$), indicating that throughout the donation, arousal scores were similar for men and women (Figure 3B).
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For cortisol, a significant interaction effect was found between time and gender \( (F(3,355)=2.821, p=.039) \). A repeated effect test indicated interaction occurred at the screening: before this, women scored overall higher than men; after this, no gender differences were apparent (Figure 3B).

**Table 2**  \( F \) and \( p \)-values for the analyses for donation-stress, arousal, and cortisol.

<table>
<thead>
<tr>
<th>Effect/contrast</th>
<th>Donation-stress</th>
<th>Arousal</th>
<th>Cortisol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main effect time</td>
<td>( F(5, 358)=66.510 )</td>
<td>( F(5, 358)=11.429 )</td>
<td>( F(3, 356)=6.660 )</td>
</tr>
<tr>
<td></td>
<td>( p&lt;.001 )</td>
<td>( p&lt;.001 )</td>
<td>( p&lt;.001 )</td>
</tr>
<tr>
<td>Linear effect time</td>
<td>( F(1, 362)=9.021 )</td>
<td>( F(1, 362)=14.072 )</td>
<td>( F(1, 358)=19.610 )</td>
</tr>
<tr>
<td></td>
<td>( p=.003 )</td>
<td>( p&lt;.001 )</td>
<td>( p&lt;.001 )</td>
</tr>
<tr>
<td>Quadratic effect time</td>
<td>( F(1, 362)=196.593 )</td>
<td>( F(1, 362)=20.093 )</td>
<td>( F(1, 358)=0.190 )</td>
</tr>
<tr>
<td></td>
<td>( p&lt;.001 )</td>
<td>( p&lt;.001 )</td>
<td>( p=.663 )</td>
</tr>
</tbody>
</table>

**Donation experience**

For donation-stress, a significant interaction effect was found for time and donation experience \( (F(5,357)=5.278, p<.001) \). A repeated effect test indicated that significant differences occurred at needle insertion, at needle uncoupling, and at the donor canteen. As Figure 3C shows, levels of donation-stress were elevated in both groups in the first part of the visit. Compared with experienced donors, first-timers start with a higher level of donation-stress, and showed a larger and higher peak during needle insertion. Scores are comparable when leaving the donation center.

For arousal, a significant interaction effect was found for time and donation experience \( (F(5,357)=5.278, p<.001) \). A repeated effect test indicated interaction occurred at the screening, at needle insertion, at needle uncoupling, and at the donor canteen. Figure 3C shows that experienced donors scored higher and less reactivity than first-timers at all these moments.

For cortisol, no significant interaction effect was found for time and donation experience \( (F(3,355)=1.354, p=.257) \), indicating that throughout the donation, cortisol levels were similar for first-time and experienced donors (Figure 3C).

**Non-acute stress**

After constructing the two groups for non-acute stress, the group low in non-acute stress comprised 140 donors. The group high in non-acute stress comprised 223.

For donation-stress, a significant interaction effect was found for time and non-acute stress \( (F(5,357)=5.628, p<.001) \). A repeated effect test indicated interaction occurred at needle uncoupling and in the donor canteen. As can be seen in Figure
3D, donors with high non-acute stress not only showed a higher level of donation-stress, but also had higher levels of donation-stress in the first part of the visit, and a steeper decline after needle insertion.

For arousal, no significant interaction effect was found for time and non-acute stress ($F(5,357)=1.425, p=.214$), indicating that throughout the donation, arousal levels of first-time and experienced donors were similar (Figure 3D).

For cortisol, a significant interaction effect was found for time and non-acute stress ($F(3,355)=3.093, p=.027$). A repeated effect test indicated interaction occurred at the screening: donors low in non-acute stress showed less cortisol reactivity than donors higher in non-acute stress (Figure 3D).

**Discussion**

Our findings provide detailed insight into psychological and hormonal stress responses during a blood donation procedure. We found a clear donation-induced response in donation-stress, which varied between the different groups of donors: women, first-time donors, and donors high on non-acute stress reported higher levels of donation-stress when compared to men, experienced donors and donors low on non-acute stress respectively. Moreover, a high and constant level of arousal was observed, indicating that donors were aroused throughout the visit. Levels of cortisol decreased during a donation when analyzing the total group, and group differences were found, indicating lower levels of cortisol up to the screening for men compared to women, and a lower cortisol response for donors low compared to high on non-acute stress.

With respect to hypothesis (1), we found lower levels of donation-stress at the end of a donation. These results largely agree with previous findings, assessing predominantly anxiety [9, 55, 56, 59–61]. In line with our hypothesis two out of three articles assessing multiple moments [57, 58], we found donation-stress peaked during needle insertion. At first sight, anxiety and donation-stress might be expected to coincide considerably, but research suggests only moderate overlap, with stress and anxiety correlating at around .55 [86, 87]. Donation-stress therefore covers a more broad range, also including nervousness and tension [14]. Our results for arousal indicate a relatively high arousal level and a small yet significant decrease during donation, also reported by Ferguson et al. [55]. Our results for cortisol partly conform with those reported by others: whereas cortisol decreased in all our donors towards the end of the donation procedure, Bellitti et al. [37] found that during a donation, serum cortisol decreased in first-time donors but not in experienced donors. The disparity may result from differences in cortisol sampling: Bellitti et al. used a fixed sampling time after donation, whereas we sampled cortisol at fixed key moments during the donation procedure.

Regarding hypothesis (2), we found that compared to men, women expressed higher levels of donation-stress and cortisol in the first part of the donation. There is no
<table>
<thead>
<tr>
<th>Effect/contrast</th>
<th>Donation-stress</th>
<th>Arousal</th>
<th>Cortisol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender*time</td>
<td>Interaction effect $F(5, 357)=2.629$ $p=.024$</td>
<td>$F(5, 357)=0.893$ $p=.486$</td>
<td>$F(3, 355)=2.821$ $p=.039$</td>
</tr>
<tr>
<td></td>
<td>Linear effect  $F(1, 361)=7.615$ $p=.006$</td>
<td>$F(1, 361)=1.020$ $p=.313$</td>
<td>$F(1, 357)=2.848$ $p=.092$</td>
</tr>
<tr>
<td></td>
<td>Quadratic effect $F(1, 361)=4.144$ $p=.043$</td>
<td>$F(1, 361)=2.116$ $p=.147$</td>
<td>$F(1, 357)=4.018$ $p=.046$</td>
</tr>
<tr>
<td></td>
<td>Interaction point Moment 4 vs 5</td>
<td>Moment 2 vs 4</td>
<td></td>
</tr>
<tr>
<td>Donation experience*time</td>
<td>Interaction effect $F(5, 357)=9.132$ $p&lt;.001$</td>
<td>$F(5, 357)=5.278$ $p&lt;.000$</td>
<td>$F(3, 355)=1.354$ $p=.257$</td>
</tr>
<tr>
<td></td>
<td>Linear effect  $F(1, 361)=0.035$ $p=.853$</td>
<td>$F(1, 361)=0.049$ $p=.824$</td>
<td>$F(1, 357)=1.243$ $p=.266$</td>
</tr>
<tr>
<td></td>
<td>Quadratic effect $F(1, 361)=32.538$ $p&lt;.001$</td>
<td>$F(1, 361)=14.440$ $p&lt;.001$</td>
<td>$F(1, 357)=2.767$ $p=.097$</td>
</tr>
<tr>
<td></td>
<td>Interaction point Moment 3 vs 4, 4 vs 5, 5 vs 6</td>
<td>Moment 2 vs 3, 3 vs 4, 4 vs 5, 5 vs 6</td>
<td></td>
</tr>
<tr>
<td>Non-acute stress*time</td>
<td>Interaction effect $F(5, 357)=5.628$ $p&lt;.001$</td>
<td>$F(5, 357)=1.425$ $p=.214$</td>
<td>$F(3, 355)=3.093$ $p=.027$</td>
</tr>
<tr>
<td></td>
<td>Linear effect  $F(1, 361)=13.602$ $p&lt;.001$</td>
<td>$F(1, 361)=2.744$ $p=.099$</td>
<td>$F(1, 357)=3.311$ $p=.070$</td>
</tr>
<tr>
<td></td>
<td>Quadratic effect $F(1, 361)=8.705$ $p=.003$</td>
<td>$F(1, 361)=2.950$ $p=.087$</td>
<td>$F(1, 357)=5.364$ $p=.021$</td>
</tr>
<tr>
<td></td>
<td>Interaction point Moment 4 vs 5, 5 vs 6</td>
<td>Moment 2 vs 4</td>
<td></td>
</tr>
</tbody>
</table>
literature investigating gender differences in stress responses in a blood donation setting, but in a non-donation setting, salivary cortisol levels indicated women were more physiologically reactive to social rejection challenges, whereas men reacted more to achievement challenges [24]. It could be argued that social acceptance, the feeling of being accepted, played a role in our finding that women were more reactive in the first part of the visit. This part of the donation procedure primarily focuses on eligibility to donate, thus potentially perceived as being accepted or not. In contrasts, men had increased cortisol levels during the last part of the donation visit, which is when the focus is on being able to successfully complete an actual donation, which might be perceived as an achievement.

Our results concerning hypothesis (3) indicate that compared to experienced donors, first-timers expressed higher levels of donation-stress in the first part of the donation. This is partly in line with previous results [9, 55, 56, 59, 59, 61]. Although a significant group effect was found for arousal (experienced donors showing higher arousal levels), both groups scored relatively high, indicating that both were clearly aroused throughout the procedure. Our finding that cortisol level was not affected by donation familiarity contrasts with the findings of Bellitti et al. [37], who found higher levels of pre-donation serum cortisol at the first donation than at the fourth donation. However, they collected serum cortisol by an additional venous blood sample instead of non-invasive saliva samples, so their donors experienced two additional venous blood samples on top of the regular phlebotomy procedure. It is conceivable that these two additional samples caused additional stress and were therefore (at least partly) responsible for the raised cortisol levels in first-time donors.

Finally, regarding hypothesis (4), we found higher scores in donation-stress for donors with high non-acute stress than for donors with low non-acute stress, which is in line with the literature [27]. Despite the significant effect for cortisol, differences on group level were below the earlier used threshold of 2.5 nmol/L [88] and are therefore considered as changes that are not relevant.

**Limitations.** Salivary cortisol has a delayed response, which hampers interpretation. In our design, to minimize stress caused by the study, we aimed for minimal invasiveness, both in types of measurements as well as interference in the routine donation procedure. We therefore assessed cortisol at the key moments, together with the questionnaire on donation-stress and arousal. The interval between consecutive measurement moments was variable between donors, mainly due to differences in waiting or relaxing time. Although we thus might have missed peaks in the cortisol response, this seems unlikely, as stable cortisol levels with small confidence intervals were found across groups and measurement moments.

The level of non-acute stress may impact upon the level of stress experienced during a donation. Non-acute stress and the number of donations were both assessed using a continuous scale, and transposed to dichotomous variables in our analyses. In future, it might be interesting to gain insight into the number of donations after which novice donors learn to cope with the donation procedure and their stress level.
Chapter 5. Psychological and hormonal stress when donating blood

### Donation-stress

- Mean donation-stress score

### Arousal

- Mean arousal score

### Cortisol

- Mean cortisol level (nmol/L)

#### Measurement moment

- Arrival
- Desk
- Screening
- Insertion
- Uncoupling
- Canteen
- Leaving

#### By gender

- Men
- Woman

#### Arousal by gender

- Men
- Woman

#### Cortisol by gender

- Men
- Woman

---

(Charts showing the stress, arousal, and cortisol levels at different moments during the donation process, with data separated by gender.)
Figures 3A to 3D Means of the different stress scores (donation-stress, arousal, and cortisol), including 95% CI error bars at key moments during a whole-blood donation, for total group (A), gender (B), donation experience (C), and non-acute stress (D).
Finally, the study itself might have had an impact on the results. For instance, the sampling of saliva using Salivettes or the sampling of psychological stress measures might be considered as unpleasant. However, the multiple evaluation moments of donation-stress and arousal, and cortisol sampling proved useful as we showed a non-linear relationship between the successive measurement moments. Measuring stress, e.g. anxiety, only before and after the phlebotomy therefore seriously underestimates the donation-stress experienced by the donor, which might be of use when evaluating the effects of anxiety on return behavior or vasovagal responses.

To conclude, we found a donation-induced effect on the level of donation-stress, which was apparent, although to some extent different, among different donor groups. Although absolute levels of donation-stress were low, we found a clear peak during needle insertion. Moreover, we found distinct differences between groups, most explicitly between first-time and experienced donors. Despite some small group differences, all donors indicated a high level of arousal. Differences in the levels of cortisol were small and therefore non-relevant. To our knowledge, ours is the first study to systematically evaluate donation-induced stress at multiple key moments in the donation procedure. We have also provided new insights into the course of the stress reaction itself. Although phlebotomy remains necessary, blood bank personnel may benefit from the scientific evidence that different donors respond differently to the various components of a blood donation procedure. However, our research needs further replication to gain more insight into the relevance of the stress response, e.g. concerning the need for stress reduction or the effects of increased stress on return behavior, and into the group differences found, e.g. by evaluating interaction effects between gender and donation experience. Moreover, also physiological stress responses might be involved, as well as stress-induced effects on coagulation.
CHAPTER 6

Physiological stress reactions during a blood donation

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Submitted
Abstract

Background: Donating blood is associated with increased psychological stress. This study investigates whether a blood donation induces physiological stress and if response patterns differ by gender, donation experience, and non-acute stress.

Study design and methods: In 372 donors, physiological stress [blood pressure, pulse rate, pulse rate variability (PRV)] was measured at seven moments during routine donation. PRV was assessed using time domain [root mean square of successive differences (RMSSD)] and frequency domain [high frequency (HF) and low frequency (LF) power] parameters. Non-acute stress was assessed by questionnaire. Shape and significance of time course patterns were assessed by fitting multilevel models for each stress measure and comparing men and women, first-time and experienced donors, and donors with high and low levels of non-acute stress.

Results: Significant response patterns were found for all stress measures, where levels of systolic blood pressure ($F(1,1315)=24.2$, $p<.001$), RMSSD ($F(1,1315)=24.2$, $p<.001$), LF ($F(1,1627)=14.1$, $p<.001$) and HF ($F(1,1624)=34.0$, $p<.001$) increased towards needle insertion and then decreased to values lower than when arriving at the donation center. Diastolic blood pressure ($F(1,1326)=50.9$, $p<.001$) increased and pulse rate ($F(1,1393)=507.4$, $p<.001$) showed a U-shaped curve. Significant group effects were found, i.e. higher systolic blood pressure/pulse rate in women; higher pulse rate in first-time donors; higher RMSSD at arrival and from screening until leaving in first-time donors; and higher LF and HF in first-time donors.

Conclusion: This study shows an increase in physiological stress related to needle insertion, followed by a decrease when leaving the donation center. Some group effects were also found.
Giving blood: donor stress and hemostasis

Introduction

Although blood donation is reported to elicit positive feelings, such as satisfaction [8], there is also evidence for the presence of a donation-induced psychological and hormonal stress response, e.g. increased pre-donation anxiety and higher levels of pre-donation serum cortisol in first-time compared to experienced donors [9, 55, 57, 58, 62]. Common, context-independent physiological stress responses include increased heart rate or pulse rate [18], increased systolic and diastolic blood pressure [19, 20, 32], and changes in heart rate variability (HRV) [20, 21]. HRV and pulse rate variability (PRV) can be used to assess sympathetic activity (activity or stress) and parasympathetic activity (organ activity) [93].

Studies investigating physiological stress responses in a blood donation setting have reported conflicting evidence. For example, blood pressure is reported to be stable [37, 94–96], to decrease [61, 94, 97, 98], or to increase [99, 100]. In addition, heart rate is reported to decrease from pre- to post-donation [37], to be stable [94–96, 98], or to increase [97, 100]. Concerning HRV, studies have reported no change in overall HRV [97, 98], a decrease in the parasympathetic HRV component [96, 97, 99, 101], and an increase in the sympathetic HRV component [96, 97, 99]. However, a decrease in the sympathetic component from pre- to post-donation was also reported [101]. Importantly, most of these studies assessed physiological stress measures at only two moments during the donation procedure, i.e. before and after phlebotomy [37, 61, 94–96, 98–100]. Only two studies performed more detailed analysis of a blood donation procedure, indicating non-linear responses in HRV parameters [97, 101]: data from the study by Middleton et al. indicates a quadratic effect in the low-frequency (LF) domain, levels decreasing from pre-to post-phlebotomy, and peaking during the phlebotomy, and a cubic effect in the high-frequency (HF) domain, overall decreasing from pre-to post-phlebotomy with highest levels at the first part of the phlebotomy and lowest levels at the second part; data from Yadav et al. indicates negative linear effects for these variables and a quadratic effect for overall HRV, peaking during the phlebotomy. Therefore, to date, the available evidence concerning physiological stress responses is fragmented and contradictory and, generally, only assessed on a few moments around the venipuncture.

Stress responses can be influenced by various factors, e.g. unfamiliarity with a situation [16]. In a donation setting, an increasing number of donations has been found to reduce psychological and hormonal stress responses [9, 55–62], but not physiological stress responses, e.g. heart rate and blood pressure levels [37]. In a non-donation setting, psychological and hormonal acute stress reactions are enhanced by levels of non-acute stress, i.e. the wide range of daily hassles or minor daily pressures 27, whereas the effect of gender is inconsistent [24–26]. Within the donation setting, differences between men and women were found in psychological stress measures such as anxiety [57], but not in hormonal and physiological stress measures, such as serum cortisol and heart rate [37]. In short, physiological stress responses during a blood donation, may be influenced by the number of previous donations, gender, and
the level of non-acute stress, although the literature to date is conflicting.

The present study examines donation-induced physiological stress response patterns, combining multiple physiological markers (blood pressure, pulse rate, PRV) at key moments during a donation procedure. The research questions were: Does blood donation induce physiological stress responses in whole-blood donors, and are there differences between men and women, first-time and experienced donors, and donors high and low on non-acute stress? The following hypotheses were formulated: 1) a blood donation induces a physiological stress response, 2) women and men show equal levels of stress, 3) first-time donors show higher levels of stress than experienced donors, and 4) donors with high levels of non-acute stress show higher levels of acute stress than donors with lower levels of non-acute stress.

Materials and methods

Participants

The current investigation is part of a study on donation-induced stress responses and their effect on donor hemostasis, called the DISTRESS study, running between October 2014 and April 2016 [102]. For this latter study, a random sample of first-time and experienced male/female donors were invited to participate. Figure 1 shows the inclusion process of the participants. The following inclusion criteria were applied: i) no use of corticosteroids (such as asthma medication), ii) no use of NSAIDS (such as aspirin) in the four days prior to blood donation, and iii) no use of beta blockers (such as atenolol).

The DISTRESS study was approved by the Medical Ethical Committee of the Academic Medical Center in Amsterdam and the Ethical Advisory Committee of Sanquin, and conducted in accordance with the principles of the Declaration of Helsinki (64th WMA General Assembly, version October 2013, Fortaleza Brazil). Reporting was done according to the Strobe Statement (version 4, October 2007).

Study procedure and protocol

A detailed description of the inclusion of the participants, as well as an overview of a routine donation procedure in the Netherlands, is provided in Hoogerwerf et al. [102]. In short, if donors were willing to participate in the study, an appointment was made for a donation. During a pre-donation talk with the researcher, the donor had the opportunity to ask questions about the study. Hereafter, informed consent was obtained.
### Exclusion 1: N=983
- N=767 for unknown reason
- N=29 for medical reason (e.g. pregnancy)
- N=22 because of travel to infectious countries
- N=51 because of time issues (i.e. lack of time)
- N=93 other reasons
- N=21 return to sender (e.g. moved)

### Exclusion 2: N=63
- N=55 for medical reasons (e.g. use of corticosteroids)
- N=5 because of travel to infectious countries
- N=3 other reasons

### Exclusion 3: N=57
- N=22 because of time issues for the donor, because they could not be contacted for an appointment, because did not show up at the appointment or for other reasons
- N=35 because of ending study

### Exclusion 4: N=27
- N=14 deferred for low HB
- N=9 deferred for medical reasons (e.g. cold)
- N=2 deferred because of travel to infectious countries
- N=1 deferred for other reasons
- N=1 removed because of using beta blocker

### N=399 participants measured

### N=372 included in analyses

---

**Figure 1** Flow chart of participant inclusion.

**Figure 2** provides an overview of the total study procedure. After obtaining informed consent, a device to register pulse rate, PRV and blood pressure (Portapres, Finapres Medical Systems, Enschede, the Netherlands) was attached to the donor and started recording (*measurement one*: after 1 min). Subsequently, height and weight were measured. Then the routine blood donation procedure started with the donor reporting at the registration desk (*measurement two*). Hereafter, the donor was screened for eligibility to donate (*measurement three*). After being directed to the donation area, needle insertion took place (*measurement four*) followed by the actual phlebotomy, with the donor in semi-recumbent position. After removal of the needle (*measurement five*), the donor was directed to the donor canteen, where the donor was requested to complete a questionnaire assessing non-acute stress (*measurement six*). After completion the donor reported to the researcher (*measurement seven*), after which the measurement device was detached.
Figure 2 Set-up of the study procedure. Key moments of a routine donation are shown above the horizontal arrow, whereas additional measurements for the current study are below the horizontal arrow. The interval between the vertical arrows roughly indicates the time between consecutive points.
Physiological stress

The Portapres uses two finger cuffs to measure finger arterial pressure under ambulant conditions [103]. Cuffs were attached to the fingers of the arm which was not used for the blood drawing, as the cuff from the donation apparatus interferes with the blood pressure as recorded by the Portapres.

PRV was assessed using time domain and frequency domain parameters [93]. In the time domain, the mean value of time intervals between successive pulse peaks (PP intervals) was calculated and used to calculate the root mean square of successive differences (RMSSD), which can be used as a precise measure of the short-term variability. Typically, lower levels of RMSSD are linked with higher pulse rate, indicating more stress or activity. In the frequency domain, a spectrum estimate was calculated for the PP interval series using discrete Fourier transform. The HF component (0.15-0.4 Hz) is thought to be mediated predominantly by the parasympathetic nervous activity, whereas the LF component (0.04-0.15 Hz) can be used to assess sympathetic efferent activity. More stress or activity is generally associated with lower levels of HF and LF. Thus, PRV was assessed by including a time domain parameter (RMSSD) and two frequency domain parameters (HF and LF).

Non-acute stress

To obtain a comprehensive impression of non-acute stress, three forms of non-acute stress were assessed: distress, work-related stress, and stress from private life. Donors’ distress was assessed using the Distress Screener [86], by rating three statements (During the past week, did you suffer from worry? During the past week, did you suffer from listlessness? During the past week, did you feel more tense?) on a three-option response scale: No (0); Sometimes (1); Regularly or more often (2). A total score, defined as Distress, was obtained by summing the three items, creating a potential range of 0-6. Work-related stress and stress from private life were assessed by rating two statements (How often have you suffered stress in the past month because of work? How often have you suffered stress in the past month in your private life?) on a five-option response scale: Never/almost never (0); Sometimes (1); Regularly (2); Often (3); Almost always/Always (4).

Demographics

Date of birth and information about lifetime number of donations were obtained from the blood bank databank (eProgesa, MAK system, France). Weight (Seca 888, Hamburg, Germany) and height (Stanley Microtoise 04-116, Besanon, France) were measured on site, and participants were requested to remove shoes, heavy clothing (e.g., jackets) and to empty their pockets. Height and weight were used to calculate body mass index (BMI) (BMI=weight/height²).
Chapter 6. Physiological stress and donating blood

Analyses

Deferred donors (e.g., because of a low hemoglobin level, a cold, or the use of drugs) were excluded from the analyses.

Physiological stress

After transferring the data to the computer, data were processed using the standard software package Beatscope (version 1.1a). Two software packages were used to analyze the data. A detailed log was kept throughout the complete procedure. Around each of the defined key moments, 30 s of data were used for the analyses, i.e. 15 s before and 15 s thereafter. Thereby, a visual inspection of the data was performed to assess reliability and representativeness. In case a shift from the original moment was needed (e.g. because it included too many missing values) caution was taken to limit this change.

Pulse rate and blood pressure - For pulse rate and blood pressure, visual inspection and the calculation of a mean was performed using a custom-written Matlab code (details available on request). These analyses were performed by a trained medical student and randomly checked by MH.

Pulse rate variability - The Kubios HRV analysis software version 2.2 (Kubios Ltd., Kuopio, Finland) was used to perform PRV analyses [93]. The smoothness priors method was applied (with a smoothing parameter of 500) to remove the very low frequency components (frequencies <0.04 Hz) from the PRV data segments [104]. A visual inspection was performed to check for major artifacts. Artifact correction was applied if necessary by selecting the lowest correction level that was possible and data segments with excessive artifacts (> 5%) were excluded from analyses. All analyses were performed by MH.

Non-acute stress

A combination score was constructed, with donors scoring below or on the median for all three forms of non-acute stress assigned to the group with 'low non-acute stress'. Donors scoring above the median for one or more of the three forms of non-acute stress were assigned to the group with 'high non-acute stress'.

Statistical analyses

To assess the shape and significance of time course patterns across the seven measurement moments, multilevel models were fitted. Analyses were performed using SPSS version 23 (IBM Corp., New York, USA). This technique allows for the estimation of individual differences in time course patterns by modeling variances of slopes and
intercepts. Moreover, it is better equipped to handle missing data or heterogeneous correlations between time points than a regular repeated measures analysis [105]. For all study variables separately, step-by-step a model was built, starting with a simple linear model with a fixed intercept and slope. Subsequently, the model was extended by i) adding a random intercept; ii) adding a quadratic effect of time; iii) adding a cubic effect of time; and iv) adding a random slope of the linear effect. Comparison between the models was done using the -2 Log Likelihood. In the best fitting, most optimal model, group effects (main and interaction) were tested by separately adding gender, donation experience and non-acute stress. A heterogeneous autoregressive covariance structure was used, which allows for higher correlations between adjacent time points than for time points that are further apart. Significance was assumed at a \( p \)-level <0.05.

**Results**

In total, 372 whole-blood donors were included in the analyses (Fig. 1). Of these, 283 (76%) had data on four or more measurements. Missing data for the stress measures occurred randomly across measurement moments, and could be attributed to noise (e.g. movement artifacts). For all stress measures, the availability of non-missing data ranged from 69-80% at each moment. Because of the skewness towards lower values (generally reported in literature [106]), both LF and HF were log-transformed: e.g. \( \text{LF (dB)} = 10 \log_{10}(\text{LF (ms}^2)) \). Donor and donation characteristics are presented in Table 1. Parameter estimates of the most optimal models are presented in Table 2. The \( F, df \) and \( p \)-values of all models are presented in Table 3, whereas only the best fitting models with main or interaction effects are described below. Figure 3 presents graphs per subgroup only when a significant main or interaction effect was found.

**Systolic blood pressure**

Means of systolic blood pressure at the different key moments across all donors ranged from 129-145 mmHg. Data showed an increase in systolic blood pressure towards needle insertion, a drop during needle uncoupling, and a small increase thereafter (cubic effect of time, \( F(1,1315)=24.2, p<.001 \)). Including donation experience and non-acute mental stress did not significantly improve the model. However, women showed a lower systolic blood pressure than men (\( F(1,335)=3.9, p=.048 \)).

**Diastolic blood pressure**

Means of diastolic blood pressure at the different key moments across all donors ranged from 74-81 mmHg. Data showed an increase in diastolic blood pressure to-
Table 1  Characteristics of the study participants.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Gender</th>
<th>Donation experience</th>
<th>Non—acute stress</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
<td>First-time Experienced</td>
</tr>
<tr>
<td>Number</td>
<td>372</td>
<td>188</td>
<td>184</td>
<td>181</td>
</tr>
<tr>
<td>Age (years)</td>
<td>40±16</td>
<td>43±16</td>
<td>37±15</td>
<td>28±9</td>
</tr>
<tr>
<td>Previous donations (n)</td>
<td>6 (0−39)</td>
<td>10 (0−52)</td>
<td>1 (0−30)</td>
<td>0 (0−0)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.5±3.9</td>
<td>24.6±3.2</td>
<td>24.5±4.4</td>
<td>23.9±3.8</td>
</tr>
</tbody>
</table>

*Presented as mean±SD.  | *Presented as median (25th−75th percentile).
wards needle insertion, a drop during needle uncoupling, and a small increase thereafter (cubic effect of time, $F(1,1326)=50.9, p<.001$). Including donation experiences, non-acute stress or gender did not significantly affect the model.

**Pulse rate**

Means of pulse rate at the different key moments across all donors ranged from 73-86 beats per minute (bpm). The course of pulse rate showed a U-shaped curve, with highest levels of pulse rate at the start and end of the donation (quadratic effect of time, $F(1,1393)=507.4, p<.001$). Step-by-step, models were constructed to assess the combined group effects, starting with a model containing gender, donation experience, and non-acute stress. This resulted in a final model containing a main effect of gender ($F(1,347)=14.0, p<.001$) and donation experience ($F(1,307)=16.0, p<.001$), and an interaction effect between donation experience and time ($F(1,286)=5.2, p=.023$). Men and experienced donors had a lower pulse rate than women and first-time donors. In addition, the difference in pulse rate between first-time and experienced donors was larger at the beginning than towards the end of the donation procedure.

**RMSSD**

Means of RMSSD at the different key moments across all donors ranged from 39.7-53.6 ms. Data showed an increase in RMSSD towards needle insertion, a drop during needle uncoupling, an increase thereafter, and a final drop when leaving the donation center (cubic effect of time, $F(1,1315)=24.2, p<.001$). Including gender or non-acute stress did not significantly affect the model. However, compared to experienced donors, first-time donors had a higher RMSSD at arrival, and from the screening until leaving the donation center, as evidenced by a significant interaction effect ($F(1,1642)=12.3, p<.001$).

**LF**

Means of LF at the different key moments across all donors ranged from 1095-2416 ms$^2$. There was a decrease in LF from arrival until reporting at the registration desk, followed by a stable level until uncoupling in first-time donors, whereas experienced donors showed a decrease during needle uncoupling. Then, LF increased when arriving at the donor canteen and then decreased again (cubic effect of time, $F(1,1627)=14.1, p<.001$). Including gender or non-acute stress did not significantly affect the model. Besides an overall higher level of LF in first-time compared to experienced donors, first-time donors had stable high levels of LF from the screening desk until needle uncoupling, whereas experienced donors showed a decrease during needle uncoupling ($F(1,1668)=8.9, p=.003$).
Table 2  Parameter estimates and 95% confidence intervals of the best fitting model for blood pressure, pulse rate and PRV.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model parameter</th>
<th>Fixed parameter 95% CI</th>
<th>Random parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$b$</td>
<td>SE $b$</td>
</tr>
<tr>
<td>SBP</td>
<td>Intercept</td>
<td>115.435</td>
<td>2.956</td>
</tr>
<tr>
<td></td>
<td>Linear</td>
<td>16.558</td>
<td>2.575</td>
</tr>
<tr>
<td></td>
<td>Quadratic</td>
<td>-4.148</td>
<td>0.721</td>
</tr>
<tr>
<td></td>
<td>Cubic</td>
<td>0.292</td>
<td>0.059</td>
</tr>
<tr>
<td></td>
<td>Gender</td>
<td>3.701</td>
<td>1.869</td>
</tr>
<tr>
<td>DBP</td>
<td>Intercept</td>
<td>63.359</td>
<td>1.643</td>
</tr>
<tr>
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<td>Linear</td>
<td>13.334</td>
<td>1.556</td>
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<td></td>
<td>Quadratic</td>
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<td></td>
<td>Cubic</td>
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<td>0.036</td>
</tr>
<tr>
<td>PR</td>
<td>Intercept</td>
<td>95.241</td>
<td>1.342</td>
</tr>
<tr>
<td></td>
<td>Linear</td>
<td>-10.343</td>
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</tr>
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<tr>
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<td>2.457</td>
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<tr>
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<td>Linear</td>
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<tr>
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<td>Quadratic</td>
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Continued on next page
Table 2 – continued from previous page

<table>
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<tr>
<th>Variable</th>
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<td>$b$</td>
<td>SE $b$</td>
</tr>
<tr>
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<td>-6.750</td>
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<tr>
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</tr>
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<td>Donation experience*linear</td>
<td>0.357</td>
<td>0.102</td>
<td>0.157</td>
</tr>
</tbody>
</table>

* SBP = systolic blood pressure; DBP = diastolic blood pressure; PR = pulse rate; RMSSD = root mean square of successive differences; LF = low-frequency power; HF = high-frequency power.
Figure 3 Means of the different stress scores (blood pressure, pulse rate, RMSSD, LF, HF), at key moments during a whole-blood donation, per subgroup if a statistically significant main or interaction effect was found.
Means of HF at the different key moments across all donors ranged from 658-1202 ms². There was a decrease in HF from arrival until reporting at the registration desk in first-time donors whereas experienced donors showed an increase, followed by an increase until needle insertion. Then, HF increased when arriving at the donor canteen and then decreased again (quadratic effect of time, $F(1,1624)=34.0, p<.001$). Including gender or non-acute stress did not significantly affect the model. Besides an overall higher level of HF in first-time compared to experienced donors, first-time donors showed a decrease in HF from arrival until reporting at the registration desk, whereas experienced donors showed an increase ($F(1,1656)=12.2, p<.001$).

### Discussion

This study provides insight into the course of physiological stress responses throughout the entire donation visit when performing a whole-blood donation. Overall the results indicate an increase in physiological stress towards needle insertion and uncoupling, followed by a decrease until leaving the donation center. Moreover, several significant group effects emerged, indicating differences between men and women, first-time and experienced donors, and between donors low and high on non-acute stress.

Our first hypothesis, stating the presence of a donation-induced physiological stress responses, was confirmed, mainly evidenced by an increase in systolic blood pressure towards needle insertion and a decrease thereafter. This response pattern is mainly in line with the literature, showing an overall decrease from pre- to post-donation values, with a large decrease during the actual phlebotomy [61, 94, 97]. As the present study is the only one to date that assesses the entire donation visit among different donor groups, this provided information on the different stress measures further in advance of the phlebotomy and for a longer period thereafter. Taking that in mind, our results on pulse rate are generally in line with the available evidence [37, 94, 95, 98]. The U-shaped response pattern of pulse rate that we found is likely to be influenced by physical activity, e.g. walking/cycling to the donation center and/or moving around during the donation visit. As a derivative from the pulse rate, RMSSD is expected to increase when pulse rate decreases, an effect that indeed took place. Accordingly, the drop in RMSSD and HF during uncoupling is most likely due to a lowered breathing rate at this point. This lowered breathing rate might be indicative of a short-term stress response during needle removal; however, this effect is probably too short to be captured by blood pressure. In addition, the decrease in HF from pre- to post-phlebotomy is in line with most similar studies [96, 97, 99], but not all [101]. Our findings of a decrease in LF are in line with one study [101], whereas other studies report an increase [97, 99] or no change at all [96]. Overall, our results on PRV parameters indicate a non-linear effect, which can also be found
Table 3  \( F \) and \( p \) for the best fitting models for blood pressure, pulse rate and PRV, by including all donors (whole group), and separately for gender, donation experience and non-acute stress.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Variable</th>
<th>Time</th>
<th>Main group effect</th>
<th>Interaction effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(linear * group)</td>
</tr>
<tr>
<td>Whole group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td></td>
<td></td>
<td>( F(1,1313)=24.0 p&lt;.001 )</td>
<td></td>
</tr>
<tr>
<td>DBP</td>
<td></td>
<td></td>
<td>( F(1,1326)=50.9 p&lt;.001 )</td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td></td>
<td></td>
<td>( F_q(1,1393)=507.8 p&lt;.001 )</td>
<td></td>
</tr>
<tr>
<td>RMSSD</td>
<td></td>
<td></td>
<td>( F_q(1,1610)=29.7 p&lt;.001 )</td>
<td></td>
</tr>
<tr>
<td>LF</td>
<td></td>
<td></td>
<td>( F(1,1380)=14.6 p&lt;.001 )</td>
<td></td>
</tr>
<tr>
<td>HF</td>
<td></td>
<td></td>
<td>( F_q(1,1428)=35.1 p&lt;.001 )</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td></td>
<td></td>
<td>( F(1,1315)=24.2 p&lt;.001 ) ( F(1,335)=3.9 p=.048 ) ( F(1,271)=0.3 p=.593 )</td>
<td></td>
</tr>
<tr>
<td>DBP</td>
<td></td>
<td></td>
<td>( F(1,1329)=51.0 p&lt;.001 ) ( F(1,334)=0.4 p=.517 ) ( F(1,296)=1.5 p=.223 )</td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td></td>
<td></td>
<td>( F_q(1,1394)=506.4 p&lt;.001 ) ( F(1,348)=14.5 p&lt;.001 ) ( F(1,291)=2.2 p=.137 )</td>
<td></td>
</tr>
<tr>
<td>RMSSD</td>
<td></td>
<td></td>
<td>No better model fit ( F(1,335)=0.6 p=.434 ) ( F(1,1648)=0.3 p=.578 )</td>
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<tr>
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<td></td>
<td></td>
<td>No better model fit ( F(1,348)=3.3 p=.069 ) ( F(1,1669)=1.4 p=.242 )</td>
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<tr>
<td>HF</td>
<td></td>
<td></td>
<td>No better model fit ( F(1,342)=2.4 p=.119 ) ( F(1,300)=1.1 p=.299 )</td>
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</tr>
<tr>
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<td></td>
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<td></td>
</tr>
<tr>
<td>SBP</td>
<td></td>
<td></td>
<td>( F(1,1315)=24.0 p&lt;.001 ) ( F(1,334)=1.4 p=.245 ) ( F(1,268)=0.2 p=.651 )</td>
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</tr>
<tr>
<td>DBP</td>
<td></td>
<td></td>
<td>( F(1,1326)=51.0 p&lt;.001 ) ( F(1,333)=2.2 p=.137 ) ( F(1,292)=0.5 p=.502 )</td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td></td>
<td></td>
<td>No better model fit ( F(1,347)=11.5 p&lt;.001 ) ( F(1,286)=5.2 p=.023 )</td>
<td></td>
</tr>
<tr>
<td>RMSSD</td>
<td></td>
<td></td>
<td>( F_q(1,1610)=29.9 p&lt;.001 ) ( F(1,1203)=0.4 p=.540 ) ( F(1,1642)=12.3 p&lt;.001 )</td>
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<tr>
<td>LF</td>
<td></td>
<td></td>
<td>( F(1,1627)=14.1 p&lt;.001 ) ( F(1,1328)=1.0 p=.326 ) ( F(1,1668)=8.9 p=.003 )</td>
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Table 3 – continued from previous page

<table>
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<tr>
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<th>Main group effect</th>
<th>Interaction effect</th>
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<td>(linear * group)</td>
</tr>
<tr>
<td>HF</td>
<td></td>
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<td>$F(1,1244)=1.4 \ p=.243$</td>
<td>$F(1,1656)=12.2 \ p&lt;.001$</td>
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<tr>
<td>Non-acute stress</td>
<td>SBP</td>
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<td>$F(1,334)=0.9 \ p=.340$</td>
<td>$F(1,266)=0.0 \ p=.938$</td>
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<tr>
<td></td>
<td>DBP</td>
<td>$F_c(1,1328)=51.0 \ p&lt;.001$</td>
<td>$F(1,333)=0.0 \ p=.935$</td>
<td>$F(1,291)=0.0 \ p=.872$</td>
</tr>
<tr>
<td></td>
<td>PR</td>
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<td>$F(1,347)=6.9 \ p=.009$</td>
<td>$F(1,287)=0.3 \ p=.565$</td>
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<td>$F(1,284)=0.0 \ p=.829$</td>
</tr>
<tr>
<td></td>
<td>HF</td>
<td>No better model fit</td>
<td>$F(1,338)=2.0 \ p=.154$</td>
<td>$F(1,294)=0.0 \ p=.938$</td>
</tr>
</tbody>
</table>

* If stated No better model fit the model found during the whole-group analyses was the most optimal model and inclusion of gender/donation experience/non-acute stress did not significantly improve this model. | b SBP=systolic blood pressure; DBP=diastolic blood pressure; PR=pulse rate; RMSSD=root mean square of successive differences; LF=low-frequency power; HF=high-frequency power | c $F_q=F$ value quadratic model; $F_c=F$ value cubic model
in other studies assessing HRV parameters in a blood donation setting [97, 101]. Thus, largely in line with literature, we demonstrated an increase in physiological stress towards needle insertion and a decrease thereafter to levels lower than that on arrival at the donation center.

Our hypothesis 2-4 handled the differences between men and women, first-time and experienced donors, and donors high and low on non-acute stress. Across all physiological measures, we observed only four significant main effects (indicating absolute differences) and four significant interaction effects (indicating different response patterns): higher systolic blood pressure and pulse rate in women; higher pulse rate in first-time donors; higher RMSSD at arrival and from screening until departure in first-time donors; and higher LF and higher HF in first-time donors. This limited number of significant effects is in contrast with our previous study, where we consistently found higher levels of psychological donation-stress in women compared to men, in first-time donors compared to experienced donors, and in donors high compared to donors low on non-acute stress [102]. However, results for pulse rate and blood pressure are comparable with those of Bellitti et al, who reported a small decrease from the first to fourth donation for heart rate, and no changes from the first to fourth donation for blood pressure [37].

Studies on stress reactivity, e.g. mental stress tests, show increases for systolic blood pressure of 20-48 mmHg, for diastolic blood pressure of 8-21 mmHg, and for a heart rate of 12-20 bpm [10, 18, 20, 32]. Most of the physiological stress responses in our study (with the exception of pulse rate) are smaller. In addition, differences due to gender and age suggest no meaningful physiological differences between men and women, first-time and experienced donors, and donors high or low on non-acute stress were found.

Study limitations — An important strength of our study was the repeated assessment of multiple stress measures. However, the different parameters of PRV are influenced by bodily activity (pulse rate and blood pressure), or speech (RMSSD and HF) which makes interpretation difficult, as the different stress measures may thus yield conflicting results. However, this method enabled to assess the physiological stress responses in considerable detail and on multiple levels. In line, the amount of missing data is within normal range, especially taking into account physiological signals are prone to errors due to several reasons such as motion artefacts. Next, based on the stress-related literature, we assessed blood pressure and heart rate indices and interpreted them primarily as a stress response [19, 21, 93], although changes in these indices might also reflect adjustments to the actual withdrawal of blood during phlebotomy. For this, it is suggested that the mild donation-induced hypovolemia is non-hypotensive, but may induce changes in autonomic tone expressed by heart rate changes and increasing blood pressure variability, indicating parasympathetic withdrawal and sympathetic activation 18. Parasympathetic activity generally decreases LF and HF and increases RMSSD [104]; however, this was not consistently shown by our results and, thus, interferes with our measurements. Future studies aimed at the assessment of routine donation-induced stress responses should be aware of the
artifacts caused by movement of the donor and try to minimize these effects by finding a match between movement restriction and not hampering the routine donation procedure, or by detailed assessment of activity during the procedure.

To conclude, despite some contradicting outcomes in the different physiological stress parameters, we found indications of increased physiological stress during a blood donation, predominantly during needle insertion. Moreover, the physiological stress responses during a blood donation appear to be comparable across men and women, first-time and experienced donors, and donors high or low on non-acute stress. This study offers new insights into the course of the physiological stress reaction itself during the entire donation procedure, whereas other studies assessed only a limited number of moments. Blood bank staff may benefit from the evidence that systolic blood pressure responds to the donation procedure. For instance, when assessing the blood pressure as part of the eligibility screening, the stress response may cause an overestimation of blood pressure. Further studies are required to verify our results and gain more insight into the relevance of the stress responses found and their potential effects on coagulation [10].
Part IV
Hemostasis
CHAPTER 7

Small but clear pro-hemostatic effects of blood donation-induced stress on donor’s hemostasis

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Eva-Maria Merz
Wim L.A.M. de Kort
Monique H.W. Frings-Dresen
Judith K. Sluiter
Jaap J. Zwaginga

Submitted
Abstract

Background: Donating blood induces stress responses in donors, e.g. increased anxiety and blood pressure. Whereas acute stress in a non-donation setting is associated with pro-hemostatic effects, such effects in blood donors have not been investigated, despite potential importance for the quality of blood products. Therefore, this study examines whether donation-induced stress has an effect on hemostatic parameters in whole-blood donors.

Study design and methods: In 372 healthy whole-blood donors, three sets of stress-related parameters were assessed at needle insertion: 1) psychological stress (i.e. donation-stress and arousal on a visual analogue scale); 2) hormonal stress, (i.e. cortisol in saliva); and 3) physiological stress (i.e. blood pressure, pulse rate, and pulse rate variability). Hemostatic parameters (PT, aPTT, fibrinogen, factor VII, factor VIII, and von Willebrand factor (vWF)) were assessed using a 5-ml citrated blood sample taken from the diversion pouch directly after needle insertion. Associations between the stress measures and hemostatic parameters were assessed using regression analyses, correcting for age, sex, BMI and blood group (O vs. non-O); here, only significant results are reported ($p<.05$).

Results: For psychological stress, a significant association was found between donation-stress and PT ($B=0.012$, $p=.025$). On the hormonal level, cortisol showed a significant positive association with fibrinogen ($B=0.168$, $p=.011$) and vWF ($B=11.376$, $p=.024$). For physiological stress, pulse rate showed a significant association with factor VII ($B=0.002$, $p=.022$), and factor VIII ($B=0.540$, $p=.003$), and a significant negative association with aPTT ($B=-0.035$, $p=.041$).

Conclusion: Overall, these results indicate that donation-induced stress responses are associated with pro-hemostatic effects on coagulation parameters and vWF.
Introduction

Although donating blood is a voluntary activity and generally not perceived to be stressful, it is known to induce stress responses. Previous studies mainly assessed psychological stress responses (e.g. anxiety) and showed a decrease in psychological stress between pre- and post-donation [9, 55, 56, 59–61]. In accordance with this, three studies that included more time points showed that levels of psychological stress remained constant [62] or increased [57, 58] from pre-donation up to needle insertion, and decreased thereafter, indicating a peak in psychological stress during needle insertion. Only one study assessed hormonal stress responses and showed that plasma cortisol decreased from pre- to post-donation in first-time donors [58]. For physiological stress, although the results are more heterogeneous, they generally suggest higher levels of physiological stress pre- compared to post-phlebotomy [37, 96, 97, 99–101].

Acute stress responses are known to influence hemostatic parameters. More specifically, although overall clotting times (prothrombin time, PT, and activated partial thromboplastin time, aPTT) remained unaltered [10], acute stress-induced increases of 5-7% were found for factor VII (FVII), 8-9% for factor VIII (FVIII), 1-5% for fibrinogen, and increases of 7-8% for von Willebrand Factor (vWF) [82, 107–109]. Despite this evidence for acute stress-induced changes in hemostatic parameters, studies assessing these effects during blood donation are lacking, even though the results may be important for donor safety, and may be expected to affect use of the final blood product.

Therefore, the present study explores whether donation-induced stress has an effect on hemostatic parameters in whole-blood donors. This was investigated by examining the immediate effects of donation-induced stress on key hemostatic parameters of the donor. Based on the literature, we hypothesized that higher levels of psychological, hormonal and/or physiological stress parameters will be associated with higher levels of FVII, FVIII, fibrinogen, and vWF, whereas levels of PT and aPTT will remain unaffected.

Materials and methods

Participants

This investigation is part of a larger study examining donation-induced stress responses and their effect on donor hemostasis (DISTRESS) [102, 110]. To ascertain study power, a sample size calculation was performed. Based on 80% power to detect a difference in means of 7% in FVII clotting activity between the stressed and non-stressed groups [111], using a two-group t-test with a 0.05 two-sided significance level, an unequal group size of 3 to 1 (non-stressed vs. stressed, unpublished data
from our department) and a common SD of 21%, an estimated number of 378 participants would be required. To allow for a 5% loss due to the analyses, the aim was to include 400 donors.

For the present study, a random sample of both first-time and experienced male/female donors were invited to participate. Excluded from participation, were donors using corticosteroids or NSAIDS in the four days prior to donation, or using beta blockers. In the Netherlands, individuals using anti-coagulation drugs are not allowed to donate blood.

![Flow chart of participant inclusion](image)

**Figure 1** Flow chart of participant inclusion.

The DISTRESS-study was conducted in accordance with the Declaration of Helsinki (64th WMA General Assembly, version October 2013, Fortaleza Brazil) and approved by the Medical Ethical Committee of the Academic Medical Center, Amsterdam (2014-070 C#2014659) and the Ethical Advisory Committee of Sanquin, Amsterdam.
Giving blood: donor stress and hemostasis

Reporting of the results is in accordance with the Strobe Statement (version 4, October 2007).

To minimize the study load on the donor, all measurements were incorporated within a routine donation procedure. Details on the invitation/inclusion of participants, an overview of a routine donation procedure in the Netherlands, and a detailed description of all the measurements involved is provided in previous papers [102, 110]. Figure 1 is a flow chart of the inclusion procedure for participants for the present study.

Study procedure

After arriving at the donation center, the donor was welcomed by the researcher. After obtaining informed consent, the Portapres (Portapres, Finapres Medical Systems BV, Enschede, the Netherlands) was attached and started recording pulse rate, pulse rate variability (PRV) and blood pressure. Height and weight were measured. Then, a routine blood donation procedure started with the donor reporting at the registration desk. Directly after needle insertion, individuals filled out a visual analogue scale (VAS) assessing stress and arousal; in addition, using a 5-ml citrated vacutainer, a blood sample was taken from the first collected blood in the diversion pouch. A saliva cortisol sample was obtained at needle removal. After completion of the donation procedure, the donor reported to the researcher to detach the Portapres. Figure 2 presents an overview of the entire study procedure.

Study parameters

Hemostatic parameters

A 5-ml citrated blood sample was taken from the diversion pouch; this ensured that no additional needle insertion was required. Stored at room temperature, the tube was sent overnight (by routine transport used for blood products) to be prepared for the analyses. Within the logistic feasibility of the present study and based on literature [10], the following set of parameters was assessed: PT (as overall marker for the extrinsic coagulation pathway); aPTT (as overall marker for the intrinsic coagulation pathway); vWF (as main platelet-vessel wall interaction mediating glycoprotein); FVIII (circulating in complex with vWF as vitamin K-independent intrinsic coagulation factor); FVII (as vitamin K-dependent extrinsic coagulation factor with short half-life); and, for the acute hemostatic phase, glycoprotein fibrinogen (as common effector protein forming the hemostatic clot) [28, 29].
Chapter 7. Blood donation-induced stress and donor hemostasis

- **Questionnaire**
- **Hormonal stress**
- **Psychological stress**
- **Physiological stress**
- **Coagulation parameters**

**Figure 2** Set-up of the study procedure. Key moments of a routine donation are shown above the horizontal arrow, whereas additional measurements for the current study are below the horizontal arrow. The interval between the vertical arrows roughly indicates the time between consecutive points.
Psychological stress

Levels of psychological stress were quantified using a VAS assessing donation-stress and arousal. The scales were a vertical bar of 120 mm, with anchors at 10 and 110 mm labeled not stressed/aroused and very stressed/aroused, respectively. Participants were instructed to answer two questions (for VAS stress: How stressed are you right now? and for VAS arousal: How aroused are you right now?) by drawing a horizontal line across the vertical line between the two anchors.

Hormonal stress

As cortisol shows its peak response at 9 min post-stressor [90], a non-invasive saliva cortisol sample was obtained at uncoupling of the needle, approximating the cortisol level at needle insertion (in this study, the average duration of donation was 10 min). Cortisol concentrations (nmol/L) were obtained by taking a non-invasive saliva cortisol sample. Individuals were asked to gently chew on a Salivette (Sarstedt, Etten-Leur, the Netherlands) for approximately 1 min. Samples were stored at -80 °C until transportation for analysis.

Physiological stress

Physiological stress measures (systolic and diastolic blood pressure, pulse rate, and PRV) were assessed with the Portapres using two cuffs to measure finger arterial pressure under ambulant conditions [103]. As the cuff from the donation apparatus may interfere with the blood pressure as recorded by the Portapres, the cuffs were attached to the fingers of the arm which was not used for drawing blood.

PRV was assessed using the time domain and frequency domain parameters [93]. In the time domain, the root mean square of successive differences (RMSSD) can be used as a precise measure of the short-term variability, where the lower levels of RMSSD are generally linked with higher levels of pulse rate, and indicate stress or activity. In the frequency domain, a spectrum estimate was calculated for the PP interval series using discrete Fourier transform. The high frequency component (HF; 0.15 - 0.4 Hz) is thought to be mainly mediated by the parasympathetic nervous system activity, whereas the LF component (LF; 0.04 - 0.15 Hz) can be used to assess sympathetic efferent activity. Lower levels of HF and LF generally indicate more stress or more activity.

Donor characteristics

Date of birth (used to calculate age) and blood group were obtained from the blood bank databank (eProgesa, MAK system, France). For weight and height measurements, participants were requested to remove shoes, heavy clothing (e.g., jackets)
Chapter 7. Blood donation-induced stress and donor hemostasis

and to empty their pockets. Weight was measured using a digital balance (Seca 888, Hamburg, Germany). Height was measured using a wall-mounted stature meter (Stanley Microtoise 04-116, Besanon, France). Body mass index (BMI) in kg/m² was calculated from height and weight (BMI=weight/height²).

Analyses

Deferred donors (e.g., because of a low hemoglobin level or a cold) were excluded from the analyses.

Hemostatic parameters

The morning after the blood sample was collected, the sample was prepared for analyses by a research technician. For this, plasma was extracted and stored at -80 °C until subject-inclusion into the study was completed. PT and aPTT were determined using a Sysmex CS-5100 (Siemens Healthcare Diagnostics, Marburg, Germany). Fibrinogen and FVII were determined using a Sysmex CA-7000 (Siemens Healthcare Diagnostics). Plasma FVIII activity and vWF antigen levels were determined using a BCS-XP (Siemens Healthcare Diagnostics) with reagents and protocols from the manufacturer, and SpectraMax Plus 96 (Molecular Devices, Sunnyvale, USA) with a homemade Elisa, respectively.

Psychological stress

From the VAS questionnaires, distance to the lowest anchor was measured in mm. For both measures, this resulted in a score between 0 (not stressed/aroused) and 100 (very stressed/aroused).

Hormonal stress

Cortisol analyses were performed by the Technische Universität Dresden, Germany. Samples were sent by courier at room temperature and stored (in Dresden) at -20 °C until analysis. After thawing, Salivettes were centrifuged, resulting in a clear supernatant of low viscosity. Salivary concentrations were measured using a high sensitivity chemiluminescence immunoassay (IBL International, Hamburg, Germany). Using a liquid handling robot, sample and reagent handling was semi-automated (Genesis, Tecan, Switzerland) and quality control samples of low, medium, and high cortisol concentrations were run on each microtiter plate assayed. The intra- and interassay coefficients for cortisol were both below 8%.
Physiological stress

After data transfer from the Portapres to the computer, data were processed using the standard software package Beatscope (version 1.1a, Finapres Medical Systems, Enschede, the Netherlands). Subsequently, two software packages (described below) were used to analyze the data. A detailed log was kept throughout the entire procedure. Around the moment of needle insertion, 30 s of data were used for the analyses, i.e. 15 s before and 15 s thereafter. For this, a visual inspection was made of the data to assess the reliability and representativeness and, when a shift from the original moment was needed (e.g. because it included too many missing values), caution was taken to limit this change.

For pulse rate and blood pressure, visual inspection and the calculation of a mean was performed using a custom-written Matlab code (details available on request). Analyses were performed by a highly trained medical student and a random check was made by MH. The software package Kubios, version 2.2 (Kubios Ltd., Kuopio, Finland) was used to perform pulse rate variability analyses. The smoothness priors method was applied (with a smoothing parameter of 500) to remove the very low frequency components (frequencies < 0.04 Hz) from the PRV data segments [104]. A visual inspection was made to check for major artifacts. If required, artifact correction was applied by selecting the lowest correction level possible, and data segments with excessive artifacts (> 5%) were excluded from the analyses. Analyses were performed by MH.

Statistical analyses

First, correlations between the study predictors (stress measures), study outcomes (hemostatic parameters) and the covariates were assessed. Covariates included age, sex and BMI (as they affect blood pressure and PRV [112–114]) as well as blood group O vs. non-O (as blood group causes differences in FVIII and vWF levels [115, 116]). Then, separate regression analyses were performed to estimate the effects of the stress measures on hemostatic parameters (analyses performed with IBM SPSS version 23). The influence of a stress measure on a hemostatic parameter was assessed by multiplying the B, as generated by the regression analyses, by the range of the stress measure and reported in percentages. Analyses were corrected for age, sex, BMI and blood group O vs. non-O. Unless stated otherwise, significance was assumed at p<.05.

Results

In total, data from 372 donors could be used in the analyses (Figure 1). Donor characteristics are presented in Table 1.
Table 1  Donor characteristics and study variables.

<table>
<thead>
<tr>
<th>Donor characteristics</th>
<th>Values</th>
<th>Range</th>
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<tr>
<td>Gender</td>
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<td>Blood group</td>
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<td>Non-O</td>
<td>239 (64)</td>
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<tr>
<td>Age</td>
<td>40±16</td>
<td>18–69</td>
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<tr>
<td>BMI</td>
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<td>15.3–45.5</td>
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<td>Previous donations</td>
<td>6 (0–39)</td>
<td>0–134</td>
</tr>
<tr>
<td>Psychological stress</td>
<td>Stress</td>
<td>36.8±24.6</td>
</tr>
<tr>
<td></td>
<td>Arousal</td>
<td>72.6±19.0</td>
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<td>Hormonal stress</td>
<td>Cortisol (nmol/L)</td>
<td>3.773 (2.265–6.304)</td>
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<td>Physiological stress</td>
<td>Systolic blood pressure (mmHg)</td>
<td>141±23</td>
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<td>Diastolic blood pressure (mmHg)</td>
<td>81±13</td>
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<td></td>
<td>Pulse rate (bpm)</td>
<td>73±12</td>
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<td></td>
<td>RMSSD (ms)</td>
<td>54±27</td>
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<tr>
<td></td>
<td>LF (ms²)</td>
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<tr>
<td></td>
<td>HF (ms²)</td>
<td>616 (286–1358)</td>
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<td>Hemostatic parameters</td>
<td>PT (s)</td>
<td>11.9±2.6</td>
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<td>aPTT (s)</td>
<td>31.5±3.4</td>
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<td>FVII (IE/ml)</td>
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<td>FVIII (%)</td>
<td>101±32</td>
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<td>Fibrinogen (g/L)</td>
<td>2.9±0.6</td>
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<td></td>
<td>vWF (%)</td>
<td>119±42</td>
</tr>
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</table>

* Presented as number (%).  |  
| b Presented as mean±SD.  |  
| c Presented as median (25th–75th percentile).  |  
| d Presented as mean±SD , 0–100 point scale.
In brief, for psychological stress during needle insertion, the mean score for donation-stress was 36.8, and for arousal was 72.6. For physiological stress, mean blood pressure was 141/81 mmHg and mean pulse rate 73 beats per minute (bpm). Because cortisol, LF and HF showed a skewness towards lower values (generally reported in literature [47, 106]), a log-transformation was applied, e.g. LF (dB) = 10 log10(LF (ms²)), and log-adjusted data are reported. For the hemostatic parameters, mean PT was 11.9 s, mean aPTT 31.5 s, mean FVII 0.9 IIE/ml, mean FVIII 101%, mean fibrinogen 2.9 g/L, and mean vWF 119%. The means of the hemostatic parameters were all within the reference ranges.

### Bivariate correlations

Table 2 presents the bivariate correlation coefficients between stress measures, hemostatic parameters, and the covariates.

For psychological stress, a significant positive correlation was found between donation-stress and PT ($r=0.117$, $p=.025$). A significant negative correlation was found between arousal and aPTT ($r=-0.106$, $p=.042$).

For hormonal stress responses, bivariate correlations between the log-transformed cortisol values and hemostatic parameters were all $p>.05$.

Finally, for physiological stress, pulse rate showed a significant positive correlation with FVII ($r=0.137$, $p=.022$), FVIII ($r=0.157$, $p=.016$) and fibrinogen ($r=0.192$, $p=.003$). Additionally, a significant negative correlation was found between HF and fibrinogen ($r=-0.131$, $p=.044$).

In summary, overall results from the bivariate, uncorrected correlations, indicate increased coagulation with higher levels of psychological and physiological stress.

### Regression analyses

Results for both the uncorrected and corrected data are presented in Table 3. In the text, the results of the models to be interpreted is presented.

For psychological stress, a significant positive association was found between donation-stress and PT ($B=0.012$, $p=.025$). Donation-stress ranged from 0 - 100. The potential donation-induced difference in PT due to donation-stress was calculated to be 1.2 s (10% change).

For hormonal stress, a significant positive association was found between cortisol and fibrinogen ($B=0.168$, $p=.011$), and between cortisol and vWF ($B=11.376$, $p=.024$). Although indicating an increased hemostasis with higher levels of cortisol, the interpretable effect sizes, as calculated for psychological stress, cannot be estimated because cortisol was log-transformed due to skewness towards lower values.
<table>
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<tr>
<th>Variable b</th>
<th>PT</th>
<th>aPTT</th>
<th>FVII</th>
<th>FVIII</th>
<th>Fibrinogen</th>
<th>vWF</th>
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<td>Donation-stress Correlation</td>
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<td><strong>0.143</strong>**</td>
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<td>Cortisol Correlation</td>
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<td>SBP b Correlation</td>
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<td>PR Correlation</td>
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<td><strong>0.137</strong>*</td>
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<td><strong>0.192</strong>**</td>
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<td>RMSSD Correlation</td>
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<td><strong>0.140</strong>*</td>
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<td>LF Correlation</td>
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<td>Sig. (2-tailed)</td>
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Continued on next page
### Table 2 – continued from previous page

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<tr>
<th>Variable</th>
<th>PT</th>
<th>aPTT</th>
<th>FVII</th>
<th>FVIII</th>
<th>Fibrinogen</th>
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<td>-0.117</td>
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<td>0.414**</td>
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*a* Significant associations indicated by an asterisk, bold font and darker cell.  
*b* SBP=systolic blood pressure; DBP=diastolic blood pressure; PR=pulse rate; RMSSD=root mean square of successive differences; LF=low-frequency power; HF=high-frequency power.
Chapter 7. Blood donation-induced stress and donor hemostasis

For physiological stress, pulse rate showed a significant positive association with FVII ($B=0.002$, $p=.022$), and FVIII ($B=0.540$, $p=.003$), and a significant negative association with aPTT ($B=-0.035$, $p=.041$). Pulse rate ranged from 50 - 113 bpm. This results in a potential donation-induced difference in FVII of 0.126 IE/ml (14%), in FVIII of 34.02% (34%), and in aPTT of 2.205 s (7%).

Thus, the overall results indicate that donation-induced stress responses are associated with increases in hemostatic parameters, indicative of increased coagulation.

Discussion

In a group of 372 healthy blood donors, significant bivariate correlations and multivariate associations were found between stress measures and hemostatic parameters. These results indicate that higher levels of donation-induced stress responses are associated with increased hemostatic parameters in healthy whole-blood donors. Increases in hemostatic parameters of 7 - 34% were found for physiological stress, and hormonal stress responses were also associated with increases in hemostatic parameters.

Donation-induced stress responses caused an increase in coagulation parameters and vWF; this is in line with others investigating, for example, acute stress following a mental stress test [82, 108, 109, 117, 118]. Associations were found between physiological and hormonal stress measures and several hemostatic parameters. In agreement with increases described in the literature for FVII, FVIII and vWF, also the negative association between aPTT and physiological stress implies a general pro-hemostatic state in the presence of higher physiological stress. In contrast, the positive association between PT and donation-stress, suggests a prolonged clotting time and, thus, a lower hemostatic state, with increased psychological stress. This might be due to differences in the stress measures assessed, and might indicate a discrepancy between the subjectivity of the psychological stress assessment (especially donation-stress) and the more objective way used in the present study to assess hormonal and physiological stress measures. In other words, donors might state that they are not stressed, whereas our findings indicate that they are stressed.

The donation-induced effect on the donor’s hemostasis has two main aspects. First, this effect might have an impact on the quality of the derived blood product. However, to our knowledge no studies have assessed this potential donation-induced effect on blood products, and our findings do not allow to draw conclusions about this. Nevertheless, the pro-hemostatic changes found are limited. This makes factors such as storage and processing (both of which might reduce or amplify the changes found) likely to be of more influence [119–121]. Additional research is needed to assess the influence of donation-induced changes in hemostatic parameters on the final blood product.

Second, our findings might also be relevant for donor safety. The relatively small
### Table 3  Regression of hemostatic parameters on different stress measures at needle insertion

The table below presents the results of regression analysis indicating the effect of different stress measures on hemostatic parameters at needle insertion. The table includes variables such as PT, aPTT, FVII, FVIII, Fibrinogen, and vWF, along with their respective coefficients (B), standard errors (SE), and significance (sig). Stress measures considered include Donation, Arousal, Cortisol, SBP, DBP, PR, RMSSD, LF, and HF.

<table>
<thead>
<tr>
<th>Variable</th>
<th>PT</th>
<th>aPTT</th>
<th>FVII</th>
<th>FVIII</th>
<th>Fibrinogen</th>
<th>vWF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>SE</td>
<td>sig</td>
<td>B</td>
<td>SE</td>
<td>sig</td>
</tr>
<tr>
<td>Donation- Uncorrected</td>
<td>0.012</td>
<td>0.005</td>
<td>0.025</td>
<td>0.010</td>
<td>0.007</td>
<td>0.168</td>
</tr>
<tr>
<td>Stress Corrected</td>
<td>0.012</td>
<td>0.006</td>
<td>0.041</td>
<td>0.004</td>
<td>0.007</td>
<td>0.567</td>
</tr>
<tr>
<td>Arousal Uncorrected</td>
<td>0.004</td>
<td>0.007</td>
<td>0.623</td>
<td>-0.019</td>
<td>0.009</td>
<td>0.042</td>
</tr>
<tr>
<td>Correction</td>
<td>0.006</td>
<td>0.007</td>
<td>0.366</td>
<td>-0.015</td>
<td>0.009</td>
<td>0.087</td>
</tr>
<tr>
<td>cortisol Uncorrected</td>
<td>0.339</td>
<td>0.332</td>
<td>0.308</td>
<td>0.671</td>
<td>0.434</td>
<td>0.123</td>
</tr>
<tr>
<td>Corrected</td>
<td>0.337</td>
<td>0.330</td>
<td>0.307</td>
<td>0.604</td>
<td>0.416</td>
<td>0.147</td>
</tr>
<tr>
<td>SBP Uncorrected</td>
<td>-0.011</td>
<td>0.007</td>
<td>0.141</td>
<td>-0.008</td>
<td>0.009</td>
<td>0.525</td>
</tr>
<tr>
<td>Corrected</td>
<td>-0.010</td>
<td>0.008</td>
<td>0.200</td>
<td>-0.005</td>
<td>0.009</td>
<td>0.569</td>
</tr>
<tr>
<td>DBP Uncorrected</td>
<td>-0.010</td>
<td>0.014</td>
<td>0.486</td>
<td>-0.018</td>
<td>0.016</td>
<td>0.253</td>
</tr>
<tr>
<td>Corrected</td>
<td>-0.010</td>
<td>0.014</td>
<td>0.467</td>
<td>-0.020</td>
<td>0.015</td>
<td>0.188</td>
</tr>
<tr>
<td>PR Uncorrected</td>
<td>-0.007</td>
<td>0.014</td>
<td>0.613</td>
<td>-0.028</td>
<td>0.017</td>
<td>0.092</td>
</tr>
<tr>
<td>Corrected</td>
<td>-0.004</td>
<td>0.015</td>
<td>0.015</td>
<td>-0.035</td>
<td>0.017</td>
<td>0.041</td>
</tr>
<tr>
<td>RMSSD Uncorrected</td>
<td>-0.001</td>
<td>0.006</td>
<td>0.824</td>
<td>0.001</td>
<td>0.008</td>
<td>0.880</td>
</tr>
<tr>
<td>Corrected</td>
<td>-0.003</td>
<td>0.007</td>
<td>0.664</td>
<td>0.000</td>
<td>0.007</td>
<td>0.964</td>
</tr>
<tr>
<td>LF Uncorrected</td>
<td>-0.004</td>
<td>0.033</td>
<td>0.912</td>
<td>-0.004</td>
<td>0.039</td>
<td>0.926</td>
</tr>
<tr>
<td>Corrected</td>
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<td>0.034</td>
<td>0.660</td>
<td>-0.014</td>
<td>0.038</td>
<td>0.714</td>
</tr>
<tr>
<td>HF Uncorrected</td>
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<td>0.035</td>
<td>0.704</td>
<td>0.041</td>
<td>0.041</td>
<td>0.316</td>
</tr>
<tr>
<td>Corrected</td>
<td>0.003</td>
<td>0.036</td>
<td>0.944</td>
<td>0.022</td>
<td>0.041</td>
<td>0.594</td>
</tr>
</tbody>
</table>

- **a** Significant associations indicated by a bold font. A corrected model should only be interpreted if the change between the uncorrected and corrected value is ≥10%. To facilitate interpretation, the model to be interpreted is indicated by a darker cell. | **b** SBP=systolic blood pressure; DBP=diastolic blood pressure; PR=pulse rate; RMSSD=root mean square of successive differences; LF=low-frequency power; HF=high-frequency power | **c** Corrected for age, sex, BMI and blood group.
increases in hemostatic parameters are similar to results from mental and physical stress tests [10], which are thought to be indicators for the onset of acute coronary symptoms. From this viewpoint, the repeated exposure to stress as a result of a blood donation (with up to 27 times a year for a plasma donor in the Netherlands) could be potentially harmful. However, such issues do not seem apparent to donors, perhaps for the following reasons. First, donors are generally ‘healthier’ compared to the average population, a phenomenon known as the healthy donor effect [122]. Second, the main mechanism behind a stress-induced increased risk for acute coronary symptoms is vascular damage due to shear stress induced by flowing blood [10]; increases in hematocrit or decreases in plasma will increase this shear stress [10]. Donors are protected from this mechanism because red blood cell donation lowers the hematocrit [123], making an increase in shear stress due to changes in hematocrit or plasma unlikely. Therefore, the potential negative health consequences of a blood donation are likely to be smaller than the health consequences due to acute mental stress tests.

Study limitations - The study itself might have caused the donor additional stress, e.g. due to the presence of the researcher, or the necessity for questionnaires, saliva sampling and measurement of pulse rate indices. This could lead to an increased stress response, thereby overestimating the effects of a standard donation procedure on hemostasis. However, by incorporating all measurements in a routine procedure the study-induced effects were minimized as much as possible. Lastly we earlier reported differences between men and women, first-time and experienced donors, and donors high and low on non-acute stress [102, 110]. Although these studies revealed some significant group effects, the stress responses were generally similar across groups; therefore, we decided not to stratify for these groups in the present study.

In conclusion, our results indicate that donation-induced stress responses induce immediate pro-hemostatic changes in both coagulation parameters and vWF. Although these changes do not appear to compromise donor safety, future studies should further explore the clinical relevance for the donor and combine this with the effects of donation-induced stress on the blood product and possible consequences for the future recipient.
Part V
General discussion
CHAPTER 8

General discussion

This thesis provides insight into the course of donation-induced stress responses, and their effects on the donor’s hemostasis. The first objective was to examine the donation-induced psychological, hormonal and physiological stress response patterns during a blood donation procedure. The second objective was to investigate the effects of the donation-induced stress response on immediate changes in hemostatic parameters. In this chapter, the main findings are presented, methodological considerations are addressed and the results are interpreted. Finally, recommendations are made for clinical practice and future research.

Main findings

Stress reactions during a blood donation and associated factors

The first research question was investigated in Part I by examining stress reactions in blood donors in a blood donation setting, as well as the associated factors (Chapter 2). Literature provided strong evidence for the occurrence of psychological stress responses and less conclusive evidence regarding hormonal and physiological stress responses in whole-blood and plasmapheresis donors. Overall, in response to the first research question, the results suggest a decrease in stress from between the start until towards the end of a routine blood donation.

Stress responses were reported to be mainly influenced by donation experience, whereby an increasing number of previous donations reduced the level of stress. Other factors triggering or enhancing the stress response were: watching high-distraction television during the phlebotomy, habitual anxiety, negative expectations, and extraversion. An avoidant and problem-focused coping style was associated with lower stress reactions, and was more frequently expressed by donors with a greater number of donations. Finally, increased ratings of pain and physical symptoms (e.g. dizziness and lightheadedness) were also associated with more stress.
Chapter 8. General discussion

Anticipatory stress response following negative experiences

The second research question (Part II) focused on whether donors experience an anticipatory stress response after having had a negative experience during their last donation (Chapters 3 and 4). In addition, the effect of the donor’s general anxiety and attitude to donating blood were examined on the association between a negative event and anticipatory stress at the following donation. Overall, the results indicate that negative experiences induce an anticipatory stress response in experienced whole-blood donors, but general anxiety and attitude to donating blood do not influence this effect among first-time donors (research question ii).

Evidence was found for an anticipatory physiological stress response (evidenced by increased blood pressure) at the subsequent visit in mainly experienced whole-blood donors who were confronted with a negative experience at their prior donation. Among the different negative experiences, fainting and dizziness showed the highest associations with increased blood pressure. Furthermore, non-donor complications (such as problems with the apparatus) were also associated with significant increases in systolic (men and women) and diastolic (women) blood pressure. Plasmapheresis donors did not show any significant changes in blood pressure related to negative donation experiences. Although differences in blood pressure were small, and not of clinical value, they indicate the presence of an anticipatory stress reaction following a negative donation experience.

Among inexperienced donors, no evidence was found that, after a previous negative experience, a donor’s general anxiety or attitude to donating blood influenced the stress response, i.e. pre-donation blood pressure.

Immediate donation-induced stress

Donation-induced psychological, hormonal stress, and physiological stress response patterns in whole-blood donors were evaluated in Part III to answer the third research question (Chapters 5 and 6). Overall, a donation-induced stress response was found to peak at needle insertion and uncoupling and decrease thereafter towards the end of the donation procedure. Differences were found related to gender, donation experience and levels of non-acute stress.

Psychological stress showed a clear donation-induced response in terms of donation-stress and arousal. Donation-stress increased towards needle insertion and declined thereafter. Moreover, higher levels of donation-stress were found in women compared to men, first-time compared to experienced donors, and donors high compared to low on non-acute stress. A high and constant level of arousal was observed, indicating that donors were aroused throughout their visit. With regard to hormonal stress, no cortisol reactivity was observed.

For physiological stress, a clear donation-induced response in blood pressure was found, again with increasing levels towards needle insertion and a decrease thereafter.
Pulse rate showed a U-shaped curve, with lowest values at needle insertion and uncoupling. The drop in parameters of the time and high frequency domains of pulse rate variability during needle uncoupling, suggest a short-term increase in stress at that moment. Also, since an increase in high frequency power is associated with increased breathing, these results suggest a lowered breathing rate during needle uncoupling, i.e. a ‘holding of the breath’ [101].

Donation-induced effects in hemostasis

For the final research question the effects of a donation-induced stress response on the levels of hemostatic parameters were assessed (Part V, Chapter 7). In general, higher levels of donation-induced hormonal and physiological stress responses were associated with higher levels of various hemostatic parameters, indicating an increased coagulation in higher-stressed donors. These effects were comparable to general stress literature.

Methodological considerations

An important strength of this thesis is the use of a combination of different research methodologies to assess donation-induced stress and its effects on the donor, i.e. a systematic review (Chapter 2), routinely administered register data from the blood bank (Chapter 3) which were combined with data from a survey study (Chapter 4), and an observational field study (Chapters 5 - 7).

Methodology

First, conducting a systematic overview of the literature (Chapter 2) provided a body of knowledge on the existing stress-related literature in the field of blood donation. Although the systematic review was designed to assess stress responses within a single routine donation procedure and, as such, did not explicitly include the concept of an anticipatory stress response after a negative donation experience, one study suggested the presence of an anticipatory stress response in relation to personality. The general concept of anticipatory stress [33] was supported, as donor return rates were decreased after experiencing a negative donation experience, such as a deferral or a vasovagal response [4, 7, 36]. Hence, we studied the possible effects of negative experiences on anticipatory stress responses, i.e. blood pressure (Chapters 3 and 4), based on routinely assessed cohort data from the blood bank as well as a survey study, and including large groups of donors. Results from the systematic review also suggested a non-linear psychological stress response. By including multiple measurement moments around a donation procedure, such an effect was indeed found in the observational field study (Chapters 5 - 7). In this thesis, use of these different
Chapter 8. General discussion

Research methodologies made it possible to assess donation-induced stress responses in a systematic and comprehensive manner.

Assessment of anticipatory stress

The database of Sanquin contains all donor screening data (e.g. blood pressure, hemoglobin) and donation information (e.g. donation success and adverse events) since 2004. Although the use of this type of data might limit the choice of parameters of interest to variables that are included in the dataset, this registry allowed to study the anticipatory stress response in large groups of donors. As a general rule, by including large numbers of participants, also very small differences between groups are likely to become statistically significant [34]. Results indeed showed several significant, albeit small positive associations between blood pressure and a number of negative experiences. However, the clinical relevance of the significant differences found (up to 3.0 mmHg systolic blood pressure) is limited, i.e. no deferral or immediate health consequences can be based on these values [124]. Nevertheless, the increase in blood pressure was considered to be an indication of anticipatory stress [33] and, thus, indicated an increased anticipatory stress in donors who had had a negative experience.

The effect of the donor’s general anxiety and attitude to donating blood was assessed in a subset of data, which was collected using data from a survey study among new donors investigating return behavior [42, 83]. In this specific group of inexperienced donors, no increased anticipatory stress response following a negative experience was found. The explanation for the disparity between both studies is twofold, including i) differences between new and experienced donors and ii) the different methods used to assess negative experiences, which is also addressed in Chapter 4. With regard to i), the explanation might be that experienced donors are prone to a cumulative effect of stress [27], or are surprised by a negative experience, or that first-time blood donors are already more stressed in the first place [80], or that they take the negative experience more in their stride. With respect to ii), in Chapter 3 regular blood bank data were used, whereas in Chapter 4 a questionnaire was used to explore to what extent donors felt they were affected by a negative experience. However, self-reporting often results in higher prevalence rates than on-site recording [8, 41]. In our study, this might be caused by reports of only the severe negative experiences on-site, whereas also less severe negative experiences might be reported by the donor. Thus, even in the absence of severe visible physical symptoms (as reported by the donor nurse), donors might perceive a negative experience as being severe (using a self-report). Either way, it is remarkable that first-time donors did not show an increased stress response following a negative donation experience, whereas this was shown by the experienced donors, as it might indicate a change in perception throughout the donor career.
Assessment of donation-induced stress

To evaluate the stress experienced during a routine donation in a real-life setting, all measurements were made during an entire routine donation procedure. In other studies, physiological stress measures were assessed in a much narrower setting, i.e. mostly examined immediately before and after phlebotomy [94, 97–99]. However, our assessment method minimized the impact of the study on the donor by limiting the number of visits and needle insertions.

A total of 1,502 invitation letters were sent out. With an overall response rate of 46%, 519 (35%) respondents were willing to participate in the study. Of these, eventually, 399 donors were measured during a whole-blood donation. Group differences were observed: first-time donors showed a response rate of 28% for men and 32% for women, whereas experienced donors showed a response rate of 73% for men and 83% for women. Response rates for new donors are lower than in previous studies at Sanquin [125], probably due to recent changes within the organization in the procedures of donor management and invitation. In addition, with respect to differences between the groups, potentially creating bias, the number of first-time donors that visit the blood bank to donate after having received an invitation card is lower than the number of experienced donors (28% for first-time donors and 59% for experienced donors; unpublished data).

The variety in measurement methods (i.e. psychological, hormonal and physiological stress measures) provided valuable information concerning the different stress responses. The assessment of multiple key moments during the donation procedure (e.g. screening and needle insertion) gave insight into the pattern of these responses. In addition, the continuous measurement of pulse rate and pulse rate variability is likely to be more reliable and less invasive than measuring this multiple times [93, 103, 126]. When comparing the various measures, the advantage of multiple assessment at key moments is evident: if levels of stress are assessed only at pre- and post-donation, the peak during needle insertion or uncoupling will then be missed. In addition, the advantage of measuring several stress measures becomes apparent: comparison between stress measures shows a clear peak in psychological stress during needle insertion (i.e. donation-stress), but also a short-term change in stress response at needle uncoupling in a subset of physiological stress measures (i.e. a drop in the parameters of the time and high-frequency domain of pulse rate variability). However, this latter effect disappears quickly and is not captured by psychological, hormonal or other physiological stress measures at this point.

The repeated measurements of psychological and hormonal stress responses, as well as the apparatus used to measure physiological stress, might have caused study-related stress in itself [16, 46]. The magnitude of this study-induced stress on top of the routine donation-induced stress is difficult to quantify but, assuming that it increases stress more or less equally across groups, might be considered non-relevant when comparing patterns between the several donor groups. Overall, assuming that the study did (to some extent) increase stress, then routine donation-induced stress
Assessment of the donor’s hemostasis

In line with the assessment of the stress measures, hemostatic parameters should (ideally) be assessed multiple times in each donor, so that the changes within an individual as a result of donation-induced stress can be observed [10, 82, 107–109]. However, one challenge was that needle insertion was indicated to be the most severe stressor [58], implying that taking another blood sample would in fact represent an additional stressor. As the main goal was to assess the routine donation-induced stress response, this was not acceptable. Therefore, to overcome this problem, it was decided to assess the differences in hemostatic parameters between high and low stressed donors. However, this necessitated the inclusion of a larger sample of donors.

A second challenge was the choice of hemostatic factors of interest. A selection of potential parameters of interest was based on literature examining both basic coagulation as well as stress [10, 22, 23, 28, 29, 82, 107–109]. Subsequently, a subset of these parameters was chosen based on feasibility; however, the choice of parameters was affected by a several factors. Firstly, the choice to perform the evaluation during a routine donation already precluded a number of factors, since the way that routinely obtained blood samples were collected and stored was determined by standard operating procedures. This, for instance, includes the use of a tourniquet, which is known to influence factors related to serum [127]. Secondly, more pragmatic reasons affected the final choice of parameters, including the costs of analyses, and the fact that one collection site would be unable to provide a sufficient number of donors within a reasonable time frame. With respect to this latter issue, donors were included at two sites, i.e. Nijmegen and Utrecht. However, the blood bank laboratories are situated in Amsterdam and the routine blood bank transport (i.e. not an express service) was used for the blood samples obtained. Because some potentially relevant analyses needed to be performed very soon after obtaining a blood sample, the transport system also excluded some parameters which might have been of interest [128].

Interpretation of findings

In the General introduction of this thesis, a model was presented for the studies that focused on whether a donation induces stress responses and how these responses might affect the donor’s hemostasis. Having discussed and reflected on the main findings and methodology, it is now discussed what there is learned from this work, based on an adjusted conceptual model (presented in Figure 1). In this figure, the
Giving blood: donor stress and hemostasis

various key elements (e.g. donation procedure, donor characteristics) are divided into specific factors (e.g. gender) and their relationships (as found in this thesis) are indicated by means of arrows. The black boxes/lines indicate the main elements and pathways (as also presented in the General introduction). The grey boxes/lines indicate more specific relationships between these factors. An arrow with a bullet head indicates that no effect was found, whereas arrows with an arrow-head indicate that an effect was found.

![Diagram](image_url)

**Figure 1** Adjusted conceptual model of the effects of donation-induced stress responses. The black boxes/lines indicate the main elements and pathways studied in this thesis. The grey boxes/lines indicate more specific relationships between a number of factors. An arrow with a bullet head indicates that no effect was found, whereas arrows with an arrow-head indicate that an effect was found.

**Donation-induced stress responses and hemostasis**

As shown throughout this thesis, a donation-induced stress response is apparent in all donor groups, with small differences between the groups. In accordance with literature assessing psychological stress [57, 58, 62], needle insertion was found to induce the most stress, as clearly shown by increases in psychological donation-stress and systolic blood pressure. In addition, results for the time domain and high frequency parameters of pulse rate variability, indicate a short-term (probably
Chapter 8. General discussion

unconscious) change in stress reactivity. This brief change in stress reactivity during needle uncoupling is immediately followed by relief, indicated by the lowered levels of psychological donation-stress at this point. In addition to this overall donation-induced stress response, donor characteristics were also found to affect the stress responses.

In the General introduction to this thesis, two donors were introduced: *Ms. R.* and *Mr. B.*. Would it be possible to predict their stress response based on the findings in this thesis, as well as the meaning of their responses? In short: *Ms. R.* was a healthy young woman, with little experience with donations, who had recently fainted during her previous donation. In contrast, *Mr. B.* was a healthy middle-aged man, with lots of experience in donating blood; however, due to travelling, his last donation has been some time ago.

Concerning the psychological parameter donation-stress, this differed according to gender, donation experience and levels of non-acute stress. Therefore, based on the evidence emerging from this thesis, compared to *Mr. B.*, *Ms. R.* is likely to show both a larger stress response for donation-stress, as well as a higher overall stress level. For arousal, results suggest *Ms.R.* would express lower levels of arousal during the entire procedure compared to *Mr. B.*, but they will both express reasonably high levels and be aroused throughout the donation procedure. These results are in line with earlier studies, indicating a decrease in stress from pre- to post-donation [9, 55–62]. When evaluating hormonal stress, neither *Ms. R.* nor *Mr. B.* are likely to show cortisol reactivity as we found no evidence of this response. This was in contrast to studies indicating differences between first-time and experienced donors [58]; however, this might be because the latter authors also assessed serum cortisol (i.e. adding another stressor due to two extra needle insertions).

Concerning physiological stress, results in this thesis indicate potential differences between *Ms. R.* and *Mr. B.* for the different measures, whereas this type of stress was not consistently evaluated in other studies [37, 61, 94–101]. However, apart from being somewhat uncertain, the relevance of these latter findings might also be limited since the differences found in this thesis were relatively small in the light of general stress-induced differences with respect to these variables [10, 18, 20, 32].

The next step was evaluation of the effects of this donation-induced stress response on the donor’s hemostasis. As shown in Chapter 8 of this thesis, donation-induced stress caused increased coagulation by raising the level of several hemostatic parameters. The increases found were comparable to laboratory mental stress tests [10, 82, 107–109]. Interestingly, here a discrepancy is revealed between the various stress measures, i.e. whereas an increase in the psychological parameter donation-stress was associated with decreased coagulation, increased hormonal and physiological stress were more robustly associated with pro-hemostatic effects. This implies that donors might state that they are not stressed, whereas our findings indicate that they are physiologically and hormonally stressed, a phenomenon that has also been described in working professionals such as ambulance workers [63].
Taking the consequences of donation-induced stress one step further

When evaluating the donation-induced stress responses and their effects on hemostasis presented in this thesis, the first question that might arise is whether this thesis’ findings require action and from whom. The findings in this thesis might be considered relevant in two ways: for the donor, and for the quality of the obtained blood product. Over-arching is the relevance for the blood bank institute, which is on the one hand dependent on the donor, and obliged to deliver a safe product for a patient on the other hand.

Regarding safety of the blood product, no studies have explored this potential donation-induced effect on blood products; moreover, the potential effects of prolonged clotting times or increases in hemostatic factors on the quality of blood products have not yet been described before. Meanwhile, the pro-hemostatic changes found in this thesis are relatively small, implying that factors such as storage and processing (both of which might reduce or enhance the changes found) are likely to have more influence [119–121].

Concerning relevance for the donor, two main aspects emerged, i.e. the stress responses might give the donor an uncomfortable feeling [8], and might also have potential (undesirable) health effects. With respect to the health effects, the relatively small increases in hemostatic parameters found are similar to results from mental or physical stress tests, which are thought to be indicators for the onset of acute coronary symptoms [10]. From this viewpoint, the repeated exposure to stress as a result of a blood donation (with up to 5 times a year for a whole-blood donor and 27 times a year for a plasma donor in the Netherlands [129]) could be potentially harmful. However, such issues do not seem to be apparent to whole-blood donors, perhaps for the following reasons. First, there is increasing evidence for a ‘healthy donor’ effect, showing that donors are generally healthier compared to the average population [122]. Second, the main mechanism behind a stress-induced increased risk for acute coronary symptoms is vascular damage due to the so-called shear stress induced by flowing blood [10]; increases in hematocrit or decreases in plasma will increase this shear stress [10]. Donors are protected from this mechanism because red blood cell donation lowers the hematocrit [123], making an increase in shear stress due to changes in hematocrit or plasma unlikely. Therefore, the potential negative health consequences of a blood donation are likely to be smaller than the health consequences due to acute mental stress tests.

Thus, the donation-induced stress responses seem to mainly affect the donors themselves, i.e. they experience them as being uncomfortable. However, as a blood donation in the Netherlands is voluntarily, donors can choose to avoid this discomfort by ceasing to donate, as they often do when, for instance, they have had a negative experience [4, 6, 7, 36]; therefore, this item is highly relevant for the blood bank. This thesis provides evidence that not only negative experiences cause anticipatory stress in a subsequent visit among experienced donors, but also that a routine dona-
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tion procedure can cause a stress response. Vasovagal reactions are known to cause donors to refrain from donating in the future [4, 5, 7] and donation anxiety is also negatively associated with donor return [36]. As the costs of recruiting and screening new donors are high, and new donors have a higher risk of transmitting infections compared to regular donors [130–132], the retention of donors is an important factor. This thesis provides valuable information and data on: i) stress response patterns, ii) differences between the various donor groups and iii) important moments during a donation process; all these factors are highly relevant when introducing/evaluating interventions to minimize the stress experienced during a donation and, subsequently, to minimize donor loss - both for new and experienced donors.

Returning to the differences in the characteristics of the two donors, Ms. R. and Mr. B., one of the most obvious actions might involve the way both donors are received and handled at the donation site. Whereas Mr. B. is likely to be content with going through the procedure by himself, Ms. R. might require more guidance during this process. Indeed, social support is known to decrease anxiety during a donation [57]; in addition, interventions aimed at reducing vasovagal reactions, such as water loading [77], applied muscle tension [31], the use of coping strategies [133], or the intake of salt [43] have also been investigated. According to the theory of Ursin and Eriksen (the CATS, presented in the General introduction), these measures might lead to a more positive expectation of the outcome, thereby lowering the stress experienced [16].

Recommendations for practice and research

The evidence provided in this thesis is useful for researchers in the field of donor studies as well as for the general literature on stress. It also provides detailed information relevant for ’on the job’ donor physicians and nurses.

Donor physicians and nurses should be aware of the phenomenon that negative experiences are associated with increased stress levels, and should attempt to comfort the donor during the negative experience, as well as during subsequent visits. This might be achieved by detailed signals in the blood bank information system, as well as by more targeted questions about how the donor experienced the various parts of the donation procedure. The differences found in the anticipatory stress responses between experienced and inexperienced donors might mainly be caused by overall increased anticipatory stress at the first visits, also indicated by increased psychological stress in first-time compared to experienced donors. Therefore, researchers should examine the number of donations it takes until a donor becomes accustomed to donating blood, in other words, when and why this increased anticipatory stress response fades away. In addition, research should focus on whether new and existing interventions aimed at preventing and handling negative experiences are justified/necessary to preserve a pool of relaxed and returning donors.
This thesis defines a number of key moments during a routine donation (e.g. at arrival, medical screening, needle insertion and uncoupling) which are likely to cause an increase in psychological and/or physiological stress. In addition, evidence is provided for an effect of donor characteristics, i.e. gender, donation experience, and levels of non-acute stress. This information is important to help decide when to comfort specific groups of donors at certain stages in a donation procedure. Therefore, future research should focus on the most effective ways to handle the donor's stress at these specific points; in addition, the magnitude of the effect of donation-induced stress on return behavior is needed to be assessed, as increased stress is likely to prevent individuals from donating again.

Since routine donation-induced changes in blood pressure and pulse rate do occur, they may lead to an overestimation of genuine blood pressure or pulse rate during the medical screening. Both donor physicians and blood banks should be aware of these phenomena, and question the use (regarding both reliability and relevance) of discrete blood pressure assessment during the medical screening.

Finally, as this thesis has provided the most detailed exploration of donation-induced stress until now, more research is needed to replicate and extrapolate the present findings: in particular, the different stress measures and their effects on hemostasis. Concerning the stress measures, objective evaluation of these can be achieved by, for instance, assessing the emotional stress of the donor via a smartphone or tablet camera in the near future. The effects on hemostasis might be extended by sampling multiple times during a donation procedure to establish changes within a donor, and by examining factors such as platelet function and circulating inflammatory markers [10, 71]. Although these changes do not appear to compromise donor safety, future studies should further explore the clinical relevance for the donor and combine this with the effects of donation-induced stress on the blood product and possible consequences for the future recipient of this product.
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Summary

Blood banks are obliged to care for their donors, since they rely on each donors’ voluntary gift for a wide variety of patients. In the last decades, most of the literature in the field of blood donation has focused on the treatment or prevention of negative donation experiences, the recruitment and retention of donors, or the effect of negative experiences on donor return rates. Studies investigating anxiety in donors report that some donors indicate pre-donation anxiety, suggesting that they perceive a donation as a ‘stressful’ event. This stress may influence the final blood product through changes in hemostasis. The aim of this thesis was to provide detailed insight into the course of donation-induced stress responses and their effects on the donor’s hemostasis. The following objectives were defined:

I Examination of donation-induced psychological, hormonal and physiological stress response patterns during a blood donation procedure;

II Investigation of the effects of donation-induced stress response on immediate changes in hemostatic parameters.

These two objectives resulted in the following research questions:

i What factors are associated with stress reactions in blood donors in a blood donation setting?

ii Do donors with and without a negative experience show different blood pressure levels at the pre-donation screening of the subsequent visit, and is this association influenced by the donor’s general anxiety and attitude related to donating blood?

iii Does a blood donation induce stress in whole-blood donors, and are there differences between men and women, first-time and experienced donors, and donors high or low on non-acute stress?

iv Does donation-induced stress have an immediate effect on hemostatic parameters in whole-blood donors?
Summary

Donation-induced psychological, hormonal and physiological stress responses

Based on the first objective (including research questions i - iii), this thesis provides detailed insight into the various donation-induced stress responses. The thesis started with a systematic evaluation of the literature concerning donation-induced stress (research question i). Subsequently, the anticipatory stress response as a result of a negative experience was evaluated (research question ii). In the following step, a detailed analysis was made of the donation-induced stress response (research question iii).

Stress reactions during a blood donation and associated factors

To study research question i, a systematic review of the literature (published up to June 2013) was performed to identify factors associated with psychological, hormonal and physiological stress reactions in blood donors in a blood donation setting (Chapter 2). Only ten studies have investigated stress responses during a blood donation setting; of these, nine assessed psychological stress responses (of which two also assessed physiological stress responses) and one study assessed physiological and hormonal stress responses only.

The experience of psychological stress consisted of increased pre-donation levels of anxiety, as well as fear and arousal, that declined towards the end of the donation procedure. In a study assessing hormonal stress responses during the first and fourth donation, results indicated a decrease in cortisol levels from pre- to post-donation in first-time donors, but not in experienced donors. Moreover, a decrease in pre-donation cortisol levels was observed between the first and fourth donation, indicating a decrease in anticipatory stress between donation one and four. Physiological stress reactions included higher pre-donation heart rate and blood pressure compared to post-donation.

Stress responses were found to be triggered or influenced by a number of factors. Most prominent was donation experience, whereby an increasing number of previous donations tended to reduce the level of stress. Other factors associated with higher stress reactions included watching high-distraction television during the phlebotomy, habitual anxiety, negative expectations, and extraversion. An avoidant and problem-focused coping method was associated with lower stress reactions, and was more often expressed by donors who had more prior donations. Finally, increased ratings of pain and physical symptoms, e.g. dizziness and lightheadedness, were also associated with more stress. With the exception of the number of prior donations (assessed in seven studies), each factor was found in at most two studies.

Taken together, regarding research question i, evidence was found for psychological stress responses in whole-blood donors, which was regularly affected by donation...
experience. Data on hormonal and physiological stress responses were less conclusive and did not show a universal pattern.

**Anticipatory stress response following negative experiences**

Chapters 3 and 4 deal with the second research question, i.e. assessing whether donors experience an anticipatory stress response after having had a negative experience in their last donation. In addition, the effect of the donor’s general anxiety and attitude to donating blood were assessed on the association between a negative event and anticipatory stress at the following donation.

In Chapter 3, the effect of a negative experience on pre-donation blood pressure levels during the medical screening of the subsequent visit was evaluated. In total, 248,118 (50% female) donors were included in the analyses, of whom 200,769 (51% female) were whole blood donors and 47,349 (42% female) plasmapheresis donors. Negative donation experiences were encountered by 26,380 (11% of the total, 61% female) donors. At the moment of study inclusion, only a small proportion of donors was a first-time donor (1,417 donors, 0.6% of the total). After stratification for sex, and adjusting for age and baseline blood pressure, whole-blood donors with a history of negative experiences generally had a higher blood pressure at the subsequent donation than donors without a history of negative experiences, however, this effect was not found for plasmapheresis donors. The main negative experiences associated with increased blood pressure were fainting and dizziness, resulting in a significant ($p<.05$) increase in systolic blood pressure of up to 3.0 mmHg in both men and women. Non-donor complications (e.g. problems with the apparatus) were also associated with a significant increase in systolic (men and women) and diastolic (women) blood pressure (up to 1.0 mmHg). In almost all groups, deferral was associated with a small but significant increases of (on average) 0.3 - 0.7 mmHg for both systolic and diastolic blood pressure. In conclusion, negative experiences might indeed cause an anticipatory stress response in the subsequent visit in whole-blood donors.

Such an anticipatory stress response following a negative experience might be influenced by the donor’s general attitude or anxiety related to the blood donation. Attitude (both cognitive and affective) indicates how a donor rates the donation in terms of importance and pleasantness, whereas donation anxiety indicates how a donor rates the donation in terms of fear. In Chapter 4, the impact of a donor’s general attitude or anxiety related to donating blood was assessed on the influence of a negative event at the following visit. In a group of 1,106 first-time blood donors (70% female), 128 men (38% of all men) and 504 women (65% of all women) indicated a negative experience at their first donation. In this group, no significant associations was found between negative experiences and pre-donation blood pressure at the subsequent visit. Moreover, of 48 interaction effects, four were significant. However, the effects were small and inconsistent. The following effects were found significant ($p<.05$), reporting the mean (5 to 95% CI): affective attitude: diastolic
blood pressure and deferral in men (4.0 (0.0 to 7.9)); systolic blood pressure and fatigue in women (-3.9 (-7.1 to -0.6)), cognitive attitude: systolic blood pressure and needle reactions in women (4.9 (0.6 to 9.2)), anxiety: diastolic blood pressure and deferral in men (-3.2 (-6.0 to -0.4)). This indicates that first-time donors did not show an increased anticipatory stress response following a negative experience. Moreover, no evidence was found that, after a previous negative experience, a donor’s general anxiety or attitude towards donation influenced the stress response, as measured by pre-donation blood pressure.

To answer research question ii: a group of predominantly experienced donors provided evidence that negative experiences are associated with increased blood pressure at the subsequent visit. Although differences in blood pressure were small, and not of clinical value, they indicate the presence of an anticipatory stress reaction following a negative donation experience. In a group of first-time donors, this effect was not present; moreover, this effect was not influenced by general anxiety and attitude to donating blood.

Immediate donation-induced stress

Blood donation-induced psychological and hormonal, and physiological stress response patterns in whole-blood donors were assessed in Chapters 5 and 6 (research question iii). In addition, differences between men and women, first-time and experienced donors, and donors with high and low non-acute stress, were examined in a group of 399 donors.

Chapter 5 assessed psychological and hormonal stress response patterns. After data-cleaning, 363 donors were included in the analyses. For psychological stress, a visual analogue scale with a potential range of 0 to 100 was used to assess donation-stress and arousal. A clear donation-induced response in donation-stress was found ($p<.001$), increasing towards needle insertion and declining hereafter. Although response patterns were highly similar, group differences were apparent, indicating higher levels of donation-stress in women compared to men ($p=.024$), first-time compared to experienced donors ($p<.001$), and donors high compared to low on non-acute stress ($p<.001$). Although a significant effect of time was found for arousal, overall a high and constant level was observed, indicating that donors were aroused throughout the visit ($p<.001$). Although response patterns were again highly similar, they differed between first-time and experienced donors ($p<.001$). For hormonal stress, although an overall significant response pattern was found with decreasing levels of cortisol towards the end of the donation ($p<.001$), as well as an effect of gender ($p=.039$) and levels of non-acute stress ($p=.027$), no cortisol reactivity (defined as a difference of ≥2.5 nmol/L) was observed.

The effects of physiological stress were assessed in Chapter 6, by assessing blood pressure, pulse rate and pulse rate variability (PRV). PRV was assessed using the root mean square of successive differences (RMSSD), high frequency (HF; 0.15 - 0.4 Hz)
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power, and low frequency (LF: 0.04 - 0.15 Hz) power. After data-cleaning, 372 donors were included in the analyses. In general, lower levels of RMSSD, HF and LF are indicative of more stress or activity. Significant response patterns were found for all stress measures, where levels of systolic blood pressure \( (p < .001) \), RMSSD \( (p < .001) \), LF \( (p < .001) \) and HF \( (p < .001) \) increased towards needle insertion and then decreased to values lower than when arriving at the donation center. Diastolic blood pressure increased \( (p < .001) \) and pulse rate showed a U-shaped curve, with highest values when arriving and when leaving the donation center \( (p < .001) \). Results for pulse rate and PRV are influenced by posture and speech, but RMSSD and HF indicate a lowered breathing rate during needle uncoupling, which might reflect a short-term increase in stress. Thus, overall results thus indicate increasing stress towards needle insertion and uncoupling, followed by a decrease until leaving the donation center.

The following significant group effects were found: women compared to men had a higher systolic blood pressure \( (p = .048) \) and pulse rate \( (p < .001) \); first-time compared to experienced donors had a higher pulse rate, with a decreasing difference towards the end of the donation (main effect donation experience \( p < .001 \), interaction effect \( p = .023 \)); first-time compared to experienced donors had a higher RMSSD at arrival, and from the screening until leaving the donation center (interaction effect \( p < .001 \)); first-time donors had stable high levels of LF from registration desk until uncoupling (interaction effect \( p = .003 \)); first-time donors showed a decrease in HF from arrival to the registration desk, whereas experienced donors showed an increase (interaction effect \( p < .001 \)). However, in this study, physiological stress responses were smaller than observed following a mental stress test and, despite the group effects found, response patterns for all groups were similar and overall do not indicate meaningful physiological differences between men and women, first-time and experienced donors, and donors high or low on non-acute stress.

Summarizing, in answer to research question iii, clear evidence was found for a donation-induced psychological, hormonal and physiological stress response. For the psychological parameter donation-stress, a larger response was shown for women compared to men, first-time compared to experienced donors, and donors high compared to low on non-acute stress. In addition, several group differences were found concerning physiological stress responses which, however, do not indicate meaningful differences between the groups.

**Donation-induced effects in hemostasis**

In Chapter 7, the effect of a donation-induced stress response on hemostasis was examined to answer research question iv. After data-cleaning, results of 372 donors were included in the analyses. Associations were assessed between the stress measures, i.e. i) psychological stress (i.e. donation-stress and arousal on a visual analogue scale); ii) hormonal stress, (i.e. cortisol in saliva); and iii) physiological stress (i.e. blood pressure, pulse rate, and pulse rate variability) and hemostatic parameters, i.e.
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Prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, factor VII, factor VIII, and von Willebrand factor (vWF). Concerning psychological stress, a significant association was found between donation-stress and PT \( (p = .025) \). For hormonal stress, cortisol showed significant associations with fibrinogen \( (p = .011) \), and vWF \( (p = .024) \). For physiological stress, pulse rate showed a significant positive association with factor VII \( (p = .022) \), and factor VIII \( (p = .003) \), and a significant negative association with aPTT \( (p = .041) \). In conclusion, these results indicate that donation-induced stress responses are associated with pro-hemostatic effects on coagulation parameters and vWF.

Concluding remarks

The general aim of this thesis was to provide detailed insight into donation-induced stress responses, and their effects on hemostatic parameters. The results, retrieved from a systematic literature review (Chapter 2), routinely administered register data from the blood bank (Chapter 3), combined with data from a survey study (Chapter 4), and an observational field study (Chapters 5 - 7), indicate the presence of an overall donation-induced stress response as well as an effect on the donor’s hemostasis. In short, psychological and physiological stress responses indicate a decrease in stress from pre- to post-donation, and peaking during needle insertion and uncoupling.

The data and evidence provided in this thesis are of use for researchers in the field of donor studies and the general literature on stress. The studies also provide detailed information relevant for ‘on the job’ donor nurses and physicians. Donor physicians and nurses should be aware of the phenomenon that negative experiences are associated with increased stress levels and aim to adequately comfort the donor during the negative experience, as well as during subsequent visits. Further, this thesis defined several key moments during a routine donation (e.g. at arrival, medical screening, needle insertion and uncoupling) which are likely to cause an increase in psychological and physiological stress. Thus, as routine donation-induced changes in blood pressure and pulse rate are shown to occur, they may lead to an overestimation of genuine blood pressure or pulse rate during the medical screening. Both donor physicians and blood banks should be aware of these phenomena and question the reliability and relevance of discrete blood pressure measurement during the medical screening.

Finally, additional research is needed to replicate and extrapolate the findings yielded by these studies on the different stress measures and their effects on hemostasis. As a final step, although the changes in hemostasis do not appear to compromise donor safety, future studies should further explore the clinical relevance for the donor and combine this with the effects of donation-induced stress on the blood product and the potential consequences for the future recipient.
Samenvatting

Bloedbanken zijn bij de inzameling van bloed verantwoordelijk voor het welzijn van de donoren, aangezien een donor de bloedbank voorziet van een vrijwillige gift bestemd voor een bloedtransfusie of medicijnen voor een patiënt. Onderzoek op het gebied van bloeddonatie is tot nu toe voornamelijk gericht op de behandeling of het voorkomen van een negatieve donatie ervaring zoals flauwvallen, de werving en het behoud van donoren, of het effect van een negatieve ervaring op de terugkomst van de donor bij de bloedbank. Eerder onderzoek toont aan dat donoren angst ervaren tijdens een donatie en dat lijkt er dus op dat een donatie wordt gezien als een stressvolle gebeurtenis. Stress kan een effect hebben op de hemostase, het mechanisme in het lichaam dat bloedverlies helpt voorkomen, onder meer door het vormen van een bloedstolsel. De veranderingen in hemostase onder invloed van stress kunnen van belang zijn voor het bloedproduct, maar of een bloeddonatie dit effect ook heeft is nog niet eerder onderzocht.

Het geven van bloed

Voordat er dieper wordt ingegaan op de verschillende aspecten die een rol spelen of bekend zijn bij een bloeddonatie, is het nuttig om een goed idee te hebben hoe een donatie in Nederland verloopt. Aanmelden als donor kan bijvoorbeeld via de website van Sanquin of bij een kraam bij bijvoorbeeld de huishoudbeurs. Hierna volgt een eerste bezoek, waarbij er enkele buisjes bloed worden afgenomen om de bloedgroep te bepalen en het bloed te testen op overdraagbare infecties. Als alles goed is, krijgt de donor een uitnodiging om voor de eerste keer te komen doneren. Na de eerste keer krijgt de donor een uitnodiging op het moment dat haar/zijn bloedgroep nodig is.

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begint. Deze duurt meestal 7 tot 12 minuten. Als er een eenheid bloed is afgenomen, 500 ml, wordt de afname automatisch gestopt door de machine, waarna de donor-assistent de naald verwijdert. Hierna krijgt de donor in de donorkantine wat te eten of te drinken aangeboden, waarna het bezoek is afgelopen.


Doel en onderzoeksvragen

Het doel van dit proefschrift is het onderzoeken van stressreacties tijdens een donatie, en de effecten hiervan op de hemostase van de donor. De onderzoeksdoelstellingen zijn het onderzoeken van:

I psychologische, hormonale en fysiologische stressreacties veroorzaakt door een donatie;

II de effecten van de stressreacties tijdens een donatie op de acute hemostase van de donor.

Deze onderzoeksdoelstellingen leiden tot de volgende onderzoeksvragen:

i Welke factoren hebben een relatie met stressreacties bij de donor tijdens een bloeddonatie?

ii Zijn er bij het eerstvolgende bezoek verschillen in bloeddruk tussen donoren met en zonder een negatieve donatie ervaring, en wordt dit effect beïnvloed door angst en attitude ten opzichte van het doneren van bloed?

iii Veroorzaakt een bloeddonatie stress bij bloeddonoren, en zijn er verschillen tussen mannen en vrouwen, nieuwe en ervaren donoren, en donoren met meer of minder stress door privé of werk?

iv Heeft de stress tijdens een donatie een onmiddelijk effect op hemostatische parameters bij bloeddonoren?
Stressreacties bij een bloeddonatie

De eerste onderzoeksdoelstelling geeft inzichten in verschillende stressreacties tijdens een donatie. Het proefschrift begint met een literatuuronderzoek naar stress bij een donatie (onderzoeksvraag i). Vervolgens is de stressreactie door een negatieve donatie ervaring bij een vorig bezoek onderzocht (onderzoeksvraag ii). Tot slot zijn er gedetailleerde metingen verricht naar stressreacties rondom een bloeddonatie (onderzoeksvraag iii).

Stressreacties bij bloeddonatie en factoren die deze beïnvloeden

Voor onderzoeksvraag i is in de literatuur gezocht naar psychologische, hormonale en fysiologische stressreacties in bloeddonoren, en factoren die tijdens een bloeddonatie deze stressreacties kunnen beïnvloeden (hoofdstuk 2). Er zijn tien studies gevonden: negen studies onderzochten psychologische stressreacties, waarbij twee studies ook fysiologische stressreacties onderzochten. één studie onderzocht enkel hormonale en fysiologische stressreacties.

Psychologische stress, in termen als angst en arousal, is verhoogd direct voor aanvang van een donatie. Arousal is een begrip dat zich lastig laat vertalen; het komt het meest overeen met termen als opwinding, alertheid en gejaagdheid, en in de rest van dit hoofdstuk wordt daarom de term alertheid gebruikt. In een studie naar hormonale stressreacties werd het stresshormoon cortisol gemeten voor en na de een eerste en vierde donatie. Resultaten laten zien dat cortisol daalt tijdens de eerste donatie, maar gelijk blijft tijdens de vierde donatie. Ook bleek de waarde direct voor de vierde donatie lager dan de waarde direct voor de eerste donatie. Fysiologische stressreacties, zoals hartslag en bloeddruk, zijn verhoogd bij aanvang van een donatie.

Een aantal factoren blijken stressreacties te veroorzaaken of te beïnvloeden. De belangrijkste factor die in verband wordt gebracht met hogere stressreacties is minder donaties in het verleden, een effect dat is beschreven in zeven studies. Ook zijn er een aantal psychologische en fysiologische factoren beschreven, ieder in maximaal twee studies. Psychologische factoren die in verband worden gebracht met een hogere stressreactie waren onder meer angststoornissen en extraversion, de mate waarin iemand op zoek is naar actie en andere mensen. Diverse manieren van omgaan met een situatie, zoals hoe oplossingsgericht iemand is, zijn bij een bloeddonatie gerelateerd aan lagere stressreacties en worden meer gebruikt door donoren die meerdere keren bloed hebben gegeven. Fysiologische factoren die in verband worden gebracht met hogere stressreacties zijn pijn en fysieke symptomen zoals duizeligheid en flauwvallen.

Wat betreft onderzoeksvraag i is sterk bewijs gevonden voor een psychologische stressreactie in bloeddonoren. Deze stressreactie lijkt voornamelijk te worden beïnvloed door het aantal eerdere donaties van de donor. Bewijs voor hormonale
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en fysiologische stressreacties is minder duidelijk naar voren gekomen in bestaande literatuur.

Stressreactie voorafgaand aan een donatie

In hoofdstukken 3 en 4 is onderzocht of er verschillen zijn in bovendruk (systolische bloeddruk) en onderdruk (diastolische bloeddruk) bij een volgend bezoek tussen donoren met en zonder een negatieve donatie ervaring (onderzoeksvraag ii). Ook werd onderzocht of dit effect wordt beïnvloed door angst en attitude (opstelling) ten opzichte van het doneren.

Het effect van een negatieve ervaring op de bloeddruk bij de eerstvolgende donatie is onderzocht in hoofdstuk 3. Er zijn 248.118 donoren (50% vrouw) meegenomen in de analyses: 200.769 bloeddonoren (51% vrouw) en 47.349 plasmadonoren (42% vrouw). Een klein deel van de donoren hadden nog niet eerder gedoneerd (1417 donoren, 0,6% van totaal). Negatieve donatie ervaringen zijn geregistreerd bij 26.380 donoren (11% van totaal, 61% vrouw). Analyses zijn apart uitgevoerd voor mannen en vrouwen en er is rekening gehouden met de leeftijd en de bloeddruk bij het bezoek vóór de negatieve ervaring. Bloeddonoren met een negatieve ervaring blijken een hogere bloeddruk te hebben bij het eerstvolgende bezoek dan bloeddonoren zonder een negatieve ervaring. Dit effect werd niet gevonden in plasmadonoren. De belangrijkste negatieve ervaringen die waren gerelateerd aan een verhoogde bloeddruk zijn flauwvallen, duizeligheid, tijdelijke afkeur en niet-donor gerelateerde complicaties zoals problemen met de apparatuur. Samenvattend kan worden gesteld dat negatieve donatie ervaringen zijn gerelateerd aan stressreacties bij het volgende bezoek voor donatie.

Deze stressreactie als gevolg van een negatieve donatie ervaring kan worden beïnvloed door de algemene attitude of angst ten opzichte van een bloeddonatie. Attitude geeft aan hoe belangrijk en aangenaam een donor een donatie vindt. De algemene angst ten opzichte van een donatie geeft aan hoe beangstigend een donor een donatie in het algemeen vindt. In hoofdstuk 4 is het effect onderzocht van de algemene attitude en angst op de relatie tussen een negatieve donatie ervaring en de bloeddruk bij een eerstvolgende donatie. In een groep van 1106 nieuwe bloeddonoren (70% vrouw) hadden 128 mannen (38% van alle mannen) en 504 vrouwen (65% van alle vrouwen) een negatieve ervaring bij hun eerste donatie. Er zijn geen verschillen gevonden in bloeddruk bij het eerstvolgende bezoek tussen donoren met en zonder een negatieve ervaring. Deze studie toont dus aan dat bij nieuwe donoren een negatieve donatie ervaring niet is gerelateerd aan stress bij het volgende bezoek. Ook is er geen bewijs gevonden voor een effect van de algemene attitude of angst in de relatie tussen negatieve ervaringen en bloeddruk bij een volgend bezoek.

Met betrekking tot onderzoeksvraag ii is er bewijs geleverd dat negatieve donatie ervaringen een relatie hebben met een verhoogde bloeddruk bij een eerstvolgend bezoek in een groep ervaren donoren. Hoewel verschillen in bloeddruk klein zijn, en
daardoor niet klinisch relevant, duiden zij op een stressreactie bij een volgend bezoek na een negatieve donatie ervaring. Echter, in een groep nieuwe donoren is dit effect niet gevonden, noch lijkt dit effect te worden beïnvloed door de algemene attitude of angst ten opzichte van een bloeddonatie.

**Acute stressreacties tijdens een donatie**

Het verloop van psychologische, hormonale en fysiologische stressreacties tijdens een donatie is onderzocht in **hoofdstukken 5 en 6** (onderzoeksvraag iii). Hiervoor zijn 399 volbloeddonors gevolgd tijdens een donatie, waarbij verschillende metingen zijn verricht en vragenlijsten zijn afgenomen om de diverse stressreacties te meten op meerdere momenten tijdens een donatie. Verschillen zijn onderzocht tussen mannen en vrouwen, nieuwe en ervaren donoren, en donor met veel of weinig stress door privé of werk.

In **hoofdstuk 5** is het verloop van de psychologische en hormonale stressreacties onderzocht. De gegevens van 363 donoren zijn gebruikt in de analyses. Voor psychologische stress is gebruik gemaakt van een visuele analoge schaal, die donatie-stress en alertheid meet op een schaal van 0 (geen) tot 100 (veel). Een donatie blijkt donatie-stress te beïnvloeden; het niveau van donatie-stress neemt toe tot het moment van het aanprikken, gevolgd door een daling. Hoewel dit verloop van donatie-stress vergelijkbaar is tussen groepen, zijn er groepsverschillen gevonden met hogere waarden van donatie-stress voor vrouwen in vergelijking met mannen, voor nieuwe in vergelijking met ervaren donoren en voor donoren met veel in vergelijking met weinig stress door privé of werk. Hoewel alertheid eenzelfde patroon laat zien tijdens een donatie, is er een hoog en vrij constant niveau gerapporteerd, hetgeen aangeeft dat alle donoren alert waren gedurende het gehele bezoek. Het verloop van de alertheid is vergelijkbaar, maar enigszins lager bij nieuwe dan bij ervaren donoren. Hoewel ook hormonale stress wordt beïnvloed door een donatie, was deze verandering slechts klein en niet klinisch relevant.

Het verloop van fysiologische stressreacties is onderzocht in **hoofdstuk 6**. Hierbij is bloeddruk, polsslag en polsslagvariabiliteit bepaald. Polsslagvariabiliteit is onderzocht door middel van een parameter in het tijdsdomein (het kwadratisch gemiddelde van de opeenvolgende waarden, RMSSD), en twee parameters in het frequentiedomein (de hoge frequentie component, HF, en de lage frequentie component, LF). Polsslagvariabiliteit is gedeeltelijk gekoppeld aan polsslag en een lagere polsslagvariabiliteit duidt in het algemeen op meer stress of activiteit. Voor alle stressmaten blijkt de hoogte van de reactie te veranderen tijdens een donatie, waarbij de waarden van bovendruk, RMSSD, LF en HF toenemen tot het moment van het aanprikken en vervolgens dalen tot waarden lager dan bij aankomst in het bloedbank. Onderdruk neemt toe en polsslag vertoont een U-vormige curve, met de hoogste waarden bij aankomst en het verlaten van de donatie centrum. Polsslag en polsslagvariabiliteit worden beïnvloed door de houding, spraak en activiteit, maar resultaten voor RMSSD en HF duiden op een kortdurende verhoging in stress tijdens
Samenvatting

Het verwijderen van de naald. Samenvattend wijzen de resultaten op toenemende fysiologische stress tot het aanprikken en verwijderen van de naald, gevolgd door een afname in stress tot het verlaten van het bloedbank.

Er zijn enkele verschillen gevonden tussen de groepen: vrouwen hebben in vergelijking met mannen een hogere bovendruk en polsslag; nieuwe donoren hebben in vergelijking met ervaren donoren een hogere polsslag, met een afnemend verschil tegen het einde van de donatie; nieuwe donoren hebben in vergelijking met ervaren donoren een hogere RMSSD bij aankomst en de keuring tot het verlaten van het bloedbank; nieuwe donoren hebben stabiele hoge niveaus van LF vanaf het registreren bij de ontvangstbalie tot het verwijderen van de naald; nieuwe donoren tonen een daling van de HF van aankomst tot de balie, terwijl ervaren donoren een stijging laten zien. Het is belangrijk te weten dat de gevonden fysiologische stressreacties in deze studie kleiner zijn dan in algemene stress-literatuur. Ook worden bloeddruk, polsslag en polsslagvariabiliteit beïnvloed door geslacht en leeftijd. Dit leidt tot de conclusie dat er geen relevante verschillen in fysiologische stress zijn tussen mannen en vrouwen, nieuwe en ervaren donoren of donoren met veel of weinig stress door privé of werk.

Het is belangrijk te weten dat de gevonden fysiologische stressreacties in deze studie kleiner zijn dan in algemene stress-literatuur. Ook worden bloeddruk, polsslag en polsslagvariabiliteit beïnvloed door geslacht en leeftijd. Dit leidt tot de conclusie dat er geen relevante verschillen in fysiologische stress zijn tussen mannen en vrouwen, nieuwe en ervaren donoren of donoren met veel of weinig stress door privé of werk.

Samenvattend en in antwoord op onderzoeksvraag iii wijzen de resultaten op de aanwezigheid van psychologische, hormonale en fysiologische stressreacties tijdens een donatie. Voor psychologische donatie-stress is een grotere stressreactie gevonden voor vrouwen in vergelijking met mannen, voor nieuwe in vergelijking met ervaren donoren en voor donoren met veel in vergelijking met weinig stress door privé of werk. Een aantal groepsverschillen zijn gevonden voor hormonale en fysiologische stressreacties, die echter niet duiden op relevante verschillen tussen deze groepen.

Stress door een donatie en hemostase

In hoofdstuk 7 is het effect onderzocht dat de stressreactie tijdens een donatie heeft op de hemostase van de donor (onderzoeksvraag iv). De gegevens van 372 donoren zijn gebruikt in de analyses. Door middel van regressieanalyses zijn de verbanden onderzocht tussen de stressmetingen (psychologische stress - donatie-stress en alertheid; hormonale stress - speeksel cortisol; en fysiologische stress - bloeddruk, polsslag en polsslagvariabiliteit) en hemostatische parameters in het bloed van de donor (prothrombinetijd (PT), geactiveerde partiële tromboplastinietijd (APTT), fibrinogeen, factor VII, factor VIII, von Willebrand factor (vWF)). De resultaten tonen aan dat de stress tijdens een donatie is gerelateerd aan een verhoogde stolling.
Aanbevelingen

Resultaten in dit proefschrift zijn zowel van nut voor donor-onderzoek als algemeen stress-gerelateerd onderzoek. Ook geven de resultaten gedetailleerde informatie die relevant kan zijn voor donorartsen en -assistenten. Donorartsen en -assistenten vinden in de resultaten bevestiging van het verschijnsel dat negatieve ervaringen gerelateerd zijn aan een verhoogde stress bij het volgende bezoek. Het is van belang dat zij helpen om de donor hierin te begeleiden, zowel tijdens de negatieve ervaring als bij een volgend bezoek.

Dit proefschrift definiert een aantal belangrijke stressmomenten tijdens een routine donatie, zoals bij binnenkomst, de medische keuring, het aanprikken en het verwijderen van de naald, die een toename in psychologische en/of fysiologische stress veroorzaken. Ook worden er verschillen beschreven tussen groepen donors. Dit biedt handvatten voor het ontwikkelen van gerichte interventies voor het verminderen van stress tijdens een volgende donatie.

De stijgingen in bloeddruk en polsslag tijdens de donatie kunnen bij de medische keuring leiden tot een overschatting van de bloeddruk of polsslag in rust. Deze kennis is relevant voor zowel donorartsen en -assistenten als de bloedbank, die het gebruik ter discussie kunnen stellen van routinematig verkregen data, zoals bloeddruk en polsslag.

Toekomstig onderzoek is nodig om de bevindingen met betrekking tot de omvang van de verschillende stress-maten en de effecten op hemostase te herhalen en uit te breiden. Ondanks dat de gevonden veranderingen in hemostase niet de veiligheid van de donor in gevaar lijken te brengen, kan toekomstig onderzoek zich richten op de klinische relevantie voor de donor. De gevolgen van de stress tijdens een donatie voor het uiteindelijke bloedproduct en de mogelijke gevolgen voor een toekomstige patiënt zijn andere ingangen van verder onderzoek.
About the author
Curriculum Vitae

Maurits Daniël Hoogerwerf was born on May 12, 1985 in Bennekom, the Netherlands. After completing the primary Waldorf School in Ede, he visited the Marnix College, where he completed the VWO track Nature and Health in 2005.

In 2009 he completed the study Human Kinetic Technology (The Hague University), during and after which he enrolled in the premaster Human Movement Sciences at the Vrije Universiteit Amsterdam, the Netherlands. For his master thesis, he investigated the effect of physical exercise on the release of cardiac troponin at the Department of Integrative Physiology, Radboud UMC Nijmegen, the Netherlands. He obtained his Masters degree in May 2011, and until the end of that year he worked at the Department of Integrative Physiology as assistant researcher. He was involved in several research projects such as the Nijmegen Four Days Marches and the Eindhoven marathon, both leading to international publications.

After a year exploring his ambitions, he started his PhD in January 2013 at the department of Donor Studies, Sanquin, and the Coronel Institute of Occupational Health, Academic Medical Center, Amsterdam, the Netherlands. Here, he worked until July 2017 on unravelling donation-induced stress responses and their effect on the donors hemostasis.

Maurits is married, and father of three children (six, four and one year of age). Besides being fascinated about people, health, exercise and technology, he also likes to go on bike holidays with the family.
Publications

Peer reviewed

Included in this thesis


Publications

Not included in this thesis


Conference abstracts


Giving blood: donor stress and hemostasis


Portfolio

Name PhD student: Maurits Daniël Hoogerwerf
PhD period: 2013 - 2017
Name PhD supervisor: Prof. dr. J.K. Sluiter, MBA; Prof. dr. M.H.W. Frings-Dresen

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### Giving blood: donor stress and hemostasis

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#### Teaching

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**Total** | 1471 | 53.1 |
Dankwoord
Dankwoord

Dit promotietraject voelde voor mij aan als een reis, een fietstocht door een onbekend gebied waarin ik het landschap heb leren lezen en waarderen. Een tocht waarin ik veel mooie, bijzondere, interessante, enthousiaste en verschillende mensen heb mogen ontmoeten, leren kennen, leren waarderen. Stuk voor stuk zijn het mensen die dit project samen met mij hebben helpen vormgeven en hebben gemaakt tot wat het is.

Hoewel dit werk niet leest als reisdagboek, staat het wel vol verhalen. Verhalen waarin jullie, bloeddonoren, als hoofdpersonen centraal staan. Jullie verdienen dan ook een plek voor aan in dit dankwoord. Jullie aanwezigheid en enthousiasme voor dit onderzoek heb ik fantastisch gevonden. De verhalen in de onderzoeksruimte vooraf, de (kritische) vragen, de humor: bedankt!

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De leden van de promotiecommissie, prof. Dr. Geurts, prof. Dr. Kunst, prof. Dr. Smets, prof. Dr. Roseboom en prof. Dr. Zwaginga, wil ik hartelijk bedanken voor het beoordelen van mijn proefschrift en hun bereidheid om te opponeren tijdens mijn verdediging ervan.

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dat ik meer dan één poot had om op te staan. Karin, Karen, Paul, Elze, Josian, Pieter, Pascalle, Jill, Daisy, Remco, Anne, Marloes: bedankt voor alle hulp en de gesprekken.

Karijn, Anne, Anne vD, Mireille: onvoorstelbaar wijs waren jullie toen ik nog maar net kon fietsen en begon bij donor studies. Wat een vreemd gevoel was het en wat moest ik vaak aan jullie denken toen ik ‘de nieuwe generatie’ aan mij de vragen hoorde stellen waarmee ik bij jullie aanklopte... Nienke, wat fijn om je hebben leren kennen: bedankt voor alle dagen in Nijmegen, gevuld met mooie gesprekken en ijsjes. Sem en Rosa: jullie kan ik eigenlijk niet apart van elkaar noemen. Niet alleen bedankt voor het zijn van mijn paranimfen, maar ook voor de borrels, de momenten dat ik geen idee had hoe de relativiteitstheorie ook-al-weer precies zat, of verlegen zat om kattenplaatjes. Lisa, jij hebt me creativiteit gezorgd voor de schitterende ontwerpen op en in dit proefschrift. En natuurlijk Tiffany, Bas, Joost, Tjeerd: mede-oio-ers. De oio-overleggen, uitjes en pannenkoekavonden zijn onvergetelijk, niet in de laatste plaats vanwege de bijdrage die we hebben geleverd aan de Nederlandse kaasconsumptie. Dank!


Naast alle nieuwe mensen die ik heb leren kennen heb ik natuurlijk ook steun en fietsadviezen gekregen van mensen die ik al kende voordat ik deze reis begon. Opa Maarten, gedurende mijn hele studie maar zeker ook tijdens mijn promotie heb je mij gevolgd en daarmee (soms) ook geleid met kritische vragen. Mam, de manier waarop je met (voedzame) ondersteuning kwam aanzetten als ik er aan toe was is grenzeloos. Annecatrien, Rosemarijke: zusjes, bedankt voor jullie aandacht en relativeringsmogelijkheden! Pap, opa Cor, oma Anna, jullie hebben in gedachten met mij meegegietst en daarbij die weg van commentaar voorzien. Corno, jouw werkethos en oplossingsgerichtheid speelden vaak in mijn achterhoofd, vooral tijdens alle avonden meten. Rick, bedankt voor de hulp als ik de layout niet (meer) overzag. Oma Anneke, Lucie, Hennie, Geert, zwagers en schoonzussen en iedereen die hier niet met naam genoemd staat: bedankt voor jullie warme interesse en welkome afwisseling.

En tot slot, lieve Johanneke, Samme, Mette en Rune. Jullie zijn allemaal een grote steun en bron van afleiding geweest de afgelopen 4,5 jaar. We zijn daarbij samen zowel fysiek als mentaal op reis geweest, waarbij de hemelvaarttochten met Samme, de pinkstertochten met Mette en de zomerse fietsvakanties met z’n allen echte rustmomenten waren waarin ik ten volle genoot van het samen zijn. Jullie aanwezigheid was een extra stimulans voor een heldere focus op het werk, zodat ik
thuis kon genieten van alle mooie momenten mt jullie. De uitdaging om in heldere
taal mijn onderzoek en werk te vatten was - en is - van onschatbare waarde voor mij.
Ik kijk uit naar onze nieuwe reizen!

Wat mij betreft stopt deze reis niet hier: de verte blijft trekken met nieuwe
vergezichten, nieuwe mensen en oude bekenden. Nogmaals, bedankt allemaal. Tot
ziens, tot ergens onderweg!